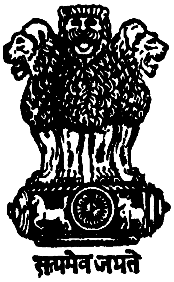

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The End.

# ON THE RELATIVE NUTRITIVE VALUE OF VARIOUS CARBOHYDRATES AND RELATED COMPOUNDS.

By

Hisasi Ariyama and Katuo Takahasi

*(From the Agricultural Chemical Laboratory of Tokio Imperial University, Komaba.)*

(Received Dec. 11 th., 1929).

The authors determined the relative nutritive value of different carbohydrates and related compounds by feeding experiments with albino rats by some different method from that of Ozaki. (Nippon Nogeikwagaku-kwaishi, **2**, 845, 1926). For this purpose the following 29 compounds were used:

Starch, Agar-Agar, Inulin, Dextrin, Glycogen, Funkan, Konjak powder, Saccharose, Maltose, Lactose, d-Glucose, d-Fructose, d-Galactose, d-Mannose, d-Glucosamine, α-Methylglucoside, l-Xylose, l-Arabinose, Dioxyacetone, d-Mannite, Glycerin, Ethyleneglycol, d-Gluconic acid, d-Saccharic acid, Mucic acid, Glyceric acid, Ethylalcohol, d, l-Alanine, l-Phenylalanine,

The basal ration used in this experiment consisted of:

| | |
|---|---|
| Meat protein | 27% |
| Butter | 70% |
| McCollum salt mixture | 3% |

(As the source of vitamin B, 0.2 g. of dry yeast was given per day per rat.)

Thus the diet was very poor on carbohydrate, neverthless the albino rats fed on this diet ad. libitum attained nearly normal growth, as shown in Chart I (A). But when it is reduced to 4.2 g. daily the animals grew up to 80~90 g. but no further. (Chart I (B))

### *Experiment 1.*

The carbohydrates tested were:

Starch, Agar-Agar, Inulin, Dextrin, Saccharose, Maltose, Lactose.

Feeding period: April to July 1928.

Average room temperature: 23C.

Young male albino rats of 40 to 45 g. were kept in a wire cage separately and were previously fed with 4.2 g. of the basal ration for about one week. Then each 1.25 g. of the samples to be tested were supplimented to it.

The rate of growth of animals during 70 days was as follows: (Chart II)

| Samples | Average gain of the body weight. | |
|---|---|---|
| | for 70 days (g.) | per day (g.) |
| Maltose | 114.0 | 1.63 |
| Starch | 95.7 | 1.37 |

| | | |
|---|---|---|
| Dextrin | 89.3 | 1.28 |
| Lactose | 89.0 | 1.27 |
| Saccharose | 81.3 | 1.26 |
| Inulin | 79.8 | 1.14 |
| Agar-Agar | 60.0 | 0.86 |
| Basal ration | 62.5 | 0.89 |

As the above result shows, the relative value is in the following order: Maltose, Starch, Dextrin, Lactose, Saccharose, Inulin and Agar-Agar; Maltose being the best. The addition of Agar-Agar had almost no effect upon the growth.

### *Experiment II.*

In the second experimint the following substances were tested:

Glucose, Fructose, Galactose, Mannose, $\alpha$-Methylglucoside, Xylose, Arabinose, Mannite, Glycerin, Ethyleneglycol. (For the control group, Starch and Dextrin were simultaneously tested.)

Feeding period: May to August 1928.

Average room temperature: 27°C.

In this experiment the animals were fed with 4.2 g. of the basal ration, as long as the animals continue to grow and when the growth was stopped 1.25 g. of each sample were added to the basal ration, then the animals began to grow up again.

Thus, the gain of the body weight after the addition of the samples may be taken as the measure of their nutritive value. The average body weight attained with 4.2 g. of the basel ration was 95 g. and the period needed for it was 41 days.

The average gain of the body weight for 40 days after adding the samples was as follows: (Chart III.)

| Samples | Average gain of the body weight. | |
|---|---|---|
| | for 40 days (g.) | per day (g.) |
| Fructose | 55.0 | 1.38 |
| Glucose | 52.5 | 1.31 |
| Starch | 52.0 | 1.30 |
| Dextrin | 51.3 | 1.28 |
| Glycerin | 49.0 | 1.23 |
| Arabinose | 35.7 | 0.89 |
| Xylose | 35.2 | 0.88 |
| Galactose | 34.2 | 0.86 |
| Mannose | 32.8 | 0.82 |
| Mannite | 25.8 | 0.65 |
| $\alpha$-Methylglucoside | 19.5 | 0.49 |

As is shown in the above table, the relative value of the samples tested is in the following order: Fructose, Glucose, Starch, Dextrin, Glycerin, Arabinose, Xylose, Galactose, Mannose, Mannite and $\alpha$-Methylglucoside, the last named being the lowest. Ethyleneglycol was found to be noxious. All the animals fed with 1.25 g. of this substance died on the next day. Contrary to the statements of some observers, Arabinose and Xylose are obviously nutritive and neither diuretic-nor diarrhetic action were observed when the amount of 1.25 g. of them were administered daily to rats. Mannose is also said to be diarrhetic, but such cases were not observed.

### *Experiment III.*

The substances tested were as follows:

Gluconic acid, Saccharic acid, Mucic acid, Glyceric acid, Glycogen, Funkan, Konjak, Glucosamine, Dioxyacetone, Alanine, Phenylalanine, Ethylalcohol. For the control group, Starch and Glucose were simultaneously tested. (Gluconic–, Saccharic–and Glyceric acid were given as Ca–salts, Mucic acid as Na, K–salt and Glucosamine as HCl–salt.)

Feeding period: January to April 1929.
Average room temperature: 16.5°C.

The experiment was carried out exactly in the same way as in the second experiment. The maximum body weight of the animals fed on 4.2 g. of the basal ration was in average 80 g.

The average gain of the body weight for 40 days after adding the samples was as follows: (Chart IV.)

| Samples | Average gain of the body weight. | |
|---|---|---|
| | for 40 days (g) | per day (g) |
| Ethylalcohol | 49.0 | 1.23 |
| Funkan | 38.7 | 0.97 |
| Glucose | 35.3 | 0.88 |
| Starch | 34.3 | 0.86 |
| Glycogen | 32.7 | 0.82 |
| Alanine | 21.8 | 0.55 |
| Konjak | 19.5 | 0.49 |
| Gluconic acid | 19.5 | 0.49 |
| Saccharic acid | 19.3 | 0.48 |
| Dioxyacetone | 7.2 | 0.18 |

As is shown in the above table, Ethylalcohol secured the best growth, followed by Funkan and Glycogen as well as Glucose and Starch. Alanine, Konjak, Gluconic acid and Saccharic acid are far inferior to Glucose, Dioxyacetone being least effective. Mucic acid, Glyceric acid, Glucosamine and Phenylalanine are found to be noxious. The animals fed with 1.25 g. of them have all died after about 2 to 25 days.

When the rats were fed with 1.25 g. of absolute Alcohol mixed with basal ration, they ate it up within 30 minutes and showed no signs of drunkness moreover, the growth was far better than on Glucose and Starch.

From these results, the albino rat seems to have relatively high resistance toward Ethylalcohol and it can assimilate fair amount of alcohol.

Assimilability of the carbohydrates tested:

At the end of the feeding period the urine of the animals excreted during 18 hours after the ingestion of the carbohydrates was collected and it was tested qualitatively for reducing substances with Benedict's reagent.

The result of the test was as follows:

| Carbohydrates | Reducing power of the urine | Carbohydrates | Reducing power of the urine |
|---|---|---|---|
| Starch | – | Glucose | – |
| Dextrin | – | Fructose | – |
| Inulin | – | Galactose | +++ |
| Agar-Agar | – | Mannose | ++ |
| Glycogen | – | α–Methylglucoside | + |
| Konjak | – | Glucosamine | ++ |
| Saccharose | – | Arabinose | +++ |
| Maltose | – | Xylose | +++ |
| Lactose | ++ | Dioxyacetone | ++ |

The urine of the animals fed with Lactose, Galactose, Mannose, α–Methylglucoside, Glucosamine, Arabinose, Xylose and Dioxyacetone reduced the reagent. Thus the above mentioned carbohydrates were excreted partially in the urine without being utilized by the animals and the low assimilability of these carbohydrates may be regarded as a caure of their relatively poor effect upon the nutritive value. In spite of its low assimilability, Lactose was found much effective on the growth of the rats especially in the early part of the feeding period.

## Summary.

(1) The basal ration used in the feeding experiments consisted of 27% Meat protein, 70% Butter and 3% McCollum salt mixture. (As the source of vitamin B, 0.2 g. of dry yeast was given per day per rat.) Male albino rats were fed with 4.2 g. of the basal ration and 1.25 g. of different kinds of carbohydrates and other substances to be tested. Observing the rate of growth of the animals, the nutritive value of the samples were compared.

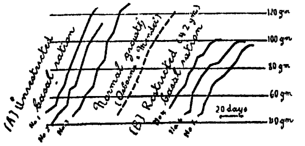
(2) The order of the nutritive value of the substances tested is as follows: As to Polysaccharide and Disaccharide, Maltose is most effective, followed by Starch, Dextrin, Lactose, Saccharose, Inulin, Glycogen and Funkan, and Konjak is far inferior to them and Agar-Agar is entirely invalid.

About Monosaccharides, Fructose and Glucose are most effective as well as Starch and Galactose, Mannose, Arabinose and Xylose come next, and Dioxyacetone and α–Methylglucoside are least effective; Glucosamine is noxious at the amount of 1.25 g. As to the effectiveness of Alcohols, Ethylalcohol seems to be most nutritive one and Glycerin follows it ranking with Starch, while Mannite is far inferior to the latter. Ethyleneglycol is poisonous, when fed with the amount of 1.25 g. of it.

As to the Acids, the nutritive value of Gluconic acid, Saccharic acid and

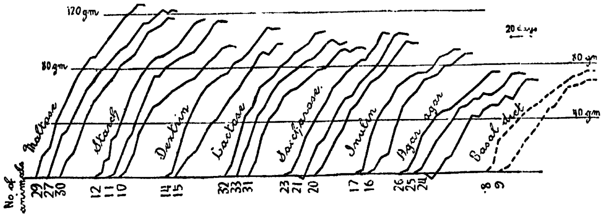
## Chart I.

The increase of body weight of rats on basal ration.



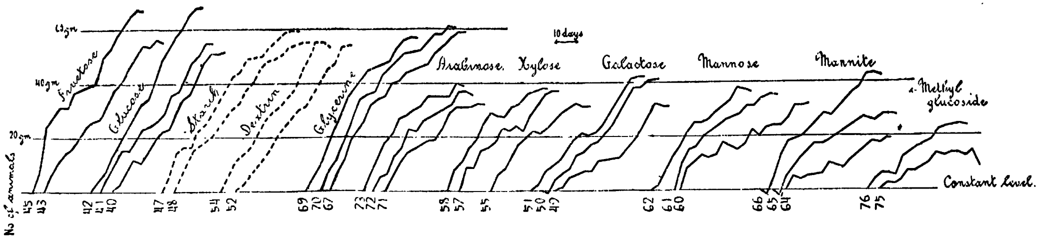
## Chart II.

Graphic records of the increase of body weight of rats after adding samples (Experiment I.).



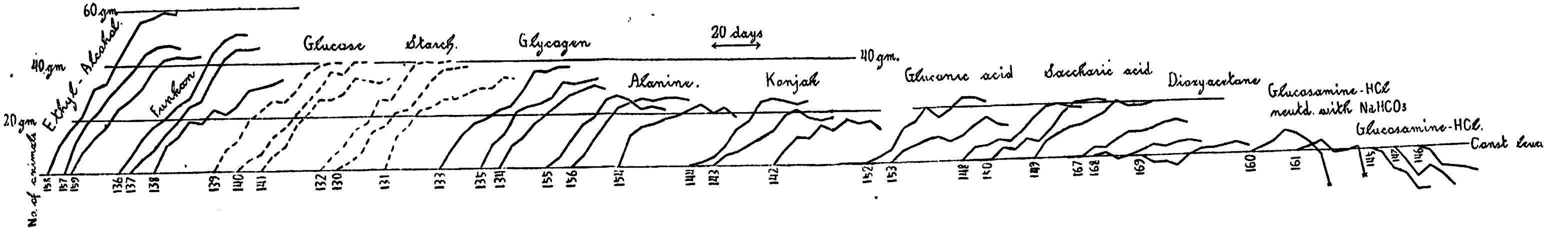
## Chart III.

Graphic records of the increase of body weight of rats after adding samples. (Experiment II.).



## Chart IV.

Graphic records of the increase of body weight of rats after adding samples. (Experiment III.).



Alanine is half of Glucose and Mucic acid, Glyceric acid and Phenylalanine are noxious at the amount of 1.25 g.

(3) Pentoses i. e., Arabinose and Xylose are found to be nutrive under our experimental condition.

(4) At the end of the feeding period, the urine of the animals was tested for reducing substances with Benedict's reagent and with the urine of Lactose, Galactose, Mannose, $\alpha$-Methylglucoside, Glucosamine, Arabinose, Xylose and Dioxyacetone positive results were obtained.

The relatively low assimilability of these carbohydrates may be regarded as a cause of their low nutritive value. In spite of its low assimilability, Lactose was much effective on the growth of the animals, especially in the early part of the feeding period.

---

# THE ASSIMILATION LIMIT OF CARBOHYDRATE

By

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*(From the Agricultural Chemical Laboratory of Tokio Imperial University, Komaba.)*

(Received Dec, 11 th., 1929).

As to the assimilability of carbohydrates, various studies have been reported by many investigators but they used mainly man, dog and rabbit as their experimental subjects.

In our previous experiment on the nutritive value of various carbohydrates, we observed that the assimilability of carbohydrates had a close relation to their nutritive value and in the present experiment, using the albino rats as the experimental animals, we measured assimilation limits of 13 kinds of carbohydrates.

## Experimental method.

The experiments were carried out chiefly after the method of Mendel and Jones (J. Biol. Chem., **43**, 491, 1920.) who had measured the tolerance of various sugars with rabbits. The ration for the albino rats consisted of meat protein 15%, starch 70%, lard 10% and McCollum salt mixture 5%.

To each 12 g. of this mixture 0.5 mg. of Biosterin and 0.01 g. of the purified Oryzanin were added.

After feeding the rats with the ration above mentioned for about 10 days, the urine was collected and tested qualitatively for reducing substances with Benedict's reagent and if the reaction was negative the rats were submitted to the experiments.

20 male albino rats weighing about 150 g. were divided into four lots and they were used repeatedly for the experiments, being submitted one lot to the experiment of one kind of carbohydrate.

The definite dosage of carbohydrate to be tested was mixed with a small portion of the ration, and was given to the rats and when this mixture was all eaten up by the animals, the remainder of the ration was supplied. After the rats ate sufficient enough amount of the diet they were divided into the wire cage provided with the funnel at the bottom and the urine voided during 18 hours was collected and tested with Benedict's reagent.

When the reaction was negative the amount of carbohydrate was increased 0.1 to 0.2 g. per 100 g. of body weight until the sugar tested appeared in the urine. According to Mendel and Jones the smallest amount of carbohydrate which resulted in glucosuria was recorded as the assimilation limit.

In the experiments of polysaccharides and disaccharides the urine was hydrolyzed with 1% HCl before it was tested for sugar.

In the experiment of Glucosamine we used Nylander's reagent in stead of Benedict's reagent, for the urine of the animals fed with Glucosamine-HCl -salt contained always reducing substance (or substances) other than the suger. As to the mixture of carbohydrates, the two components were mixed in the proportion of 1 : 1.

## Results of the experiments.

The results of the expriments were shown in Table I, II, III, and IV. The urine of the animals fed with Inulin and Dextrin reduced the reagent only after the inversion and so these two carbohydrates are seemed to be excreted in the non-reducing polysaccharide form. Saccharose may be regarded as to be excreted in the unchanged-or inverted form, because out of five animals the urine of three animals (No. 101, 102, 103.) reduced the reagent while the urine of the other two animals (100, 104.) exhibited the reducing action after inversion. The comparison of our results with that of Mendel and Jones is given in the table V. As is shown in this table, the two figures for the tolerance of Maltose, Glucose, Saccharose and Fructose are almost similar,. except that the order of Saccharose and Fructose is reversed.

As for Dextrin our average figure is 0.82 g., corresponding to half that of Mendel and Jones (1.61 g.)

In our results the assimilation limit of the mixture of sugars lies between that of two components while in the results of Mendel and Jones the tolerance for the mixture is less than that of either component (excepting for the Dextrin–glucose mixture.)

### TABLE I.

| Animal No. | Limits of assimilability per 100 g. of body weight. | | |
|---|---|---|---|
| | Galactose | Lactose | Glucose + Lactose |
| | g. | g. | g. |
| 90 | −0.10 | 0.30 | 0.20 |
| 91 | 0.10 | 0.10 | 0.20 |
| 92 | 0.10 | 0.10 | 0.30 |
| 93 | −0.10 | 0.10 | 0.40 |
| 94 | 0.10 | 0.20 | 0.20 |
| average tolerance | −0.10 or 0.10 | 0.16 | 0.26 |

(Minus signs indicate that the assimilation limit was less than figure given).

### TABLE II.

| Animal No. | Limits of Assimilability per 100 g. of body weight. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Xylose | Arabinose | Fructose | Saccharose | Saccharose + Dextrin | Saccharose + Maltose | Fructose + Glucose | Dextrin + Maltose |
| | g. | g. | g | g. | g. | g. | g. | g. |
| 100 | −0.10 | 0.10 | 0.40 | 0.80 | 0.80 | 1.10 | 1.00 | 1.40 |
| 101 | 〃 | 〃 | 0.50 | 0.70 | 0.80 | 1.00 | 0.90 | 1.40 |
| 102 | 〃 | 〃 | 0.60 | 0.80 | 0.70 | 1.20 | 1.00 | 1.20 |
| 103 | 〃 | 〃 | 0.40 | 0.70 | 0.70 | 0.70 | 1.00 | 1.20 |
| 104 | 〃 | 〃 | 0.60 | 0.80 | 0.80 | 0.90 | 1.00 | 1.40 |
| average tolerance | −0.10 | 0.10 | 0.50 | 0.76 | 0.76 | 0.98 | 0.98 | 1.32 |

### TABLE III.

| Animal No. | Limits of Assimilability per 100 g. of body weight. | | | | | | |
|---|---|---|---|---|---|---|---|
| | Mannose | Dextrin | Saccharose + Glucose | Dextrin + Glucose | Glucose | Maltose | Inulin |
| | g. | g. | g. | g. | g. | g. | g. |
| 110 | 0.20 | 0.80 | 1.00 | 0.90 | 1.10 | 1.50 | 1.50 |
| 111 | 0.20 | 0.80 | 0.80 | 0.90 | 1.00 | 1.40 | 1.50 |
| 112 | 0.30 | 0.90 | 0.80 | 1.00 | 1.10 | 1.60 | 1.50 |
| 113 | 0.30 | 0.80 | 1.20 | 1.00 | 0.90 | 1.60 | 1.60 |
| 114 | 0.40 | 0.80 | 1.00 | 1.00 | 1.10 | 1.40 | 1.70 |
| average tolerance. | 0.28 | 0.82 | 0.96 | 0.96 | 1.04 | 1.50 | 1.56 |

## TABLE IV.

| Animal No. | Limits of Assimilability per 100 g. of body weight. | |
|---|---|---|
| | Glucosamine HCl-salt | Dioxyacetone |
| | g. | g. |
| 120 | 0.30 | 0.30 |
| 121 | 0.20 | 0.20 |
| 122 | 0.40 | 0.40 |
| 123 | 0.30 | 0.30 |
| 124 | 0.30 | 0.40 |
| average tolerance | 0.30 | 0.32 |

## TABLE V.

| Carbohydrates. | Average tolerance per 100 g. of body weight. | |
|---|---|---|
| | The authors | Mendel and Jones |
| | g. | g. |
| Inulin | 1.56 | —— |
| Maltose | 1.50 | 1.71 |
| Glucose | 1.04 | 1.30 |
| Dextrin | 0.82 | 1.61 |
| Saccharose | 0.76 | 0.75 |
| Fructose | 0.50 | 0.80 |
| Dioxyacetone | 0.32 | —— |
| Glucosamine HCl-salt | 0.30 | —— |
| Mannose | 0.28 | —— |
| Lactose | 0.16 | —— |
| Arabinose | 0.10 | —— |
| Galactose | −0.10 or 0.10 | —— |
| Xylose | −0.10 | —— |
| Dextrin + Maltose | 1.32 | 1.50 |
| Saccharose + Maltose | 0.98 | —— |
| Fructose + Glucose | 0.98 | 0.75* |
| Saccharose + Glucose | 0.96 | —— |
| Dextrin + Glucose | 0.96 | 1.46 |
| Saccharose + Dextrin | 0.76 | 0.69 |
| Lactose + Glucose | 0.26 | —— |

* Mendel and Jones studied with inverted Saccharose.

# ON THE OXIDIZING ACTION OF CRYPTOMERIA-TIMBER, VESSELS OF SAKÉ AND SHŌYU.

By

MASAKAZU YAMADA

(Received Dec. 12 th., 1929.)

The properties of the alcohol oxidizing power of Cryptomeria-timber(1) were further investigated. The same action is common to some other woods.

In Cryptomeria the zone between heart-and sap-wood is generally the most powerful, the heart-wood moderate and the outer part of sap-wood, the leaf, the bark and the twig are far inferior whatever the unseasoned or not. The color of alcohol solution produced by the timber has not to do with the activity. The optimal temperature for the activity is 25 to 50°C. The timbers steamed for half an hour at 100° and also heated in the dry state for an hour at 150° lose the power. As to the effect of the reaction at $P_H$ below 9.0 the activity is gradually reduced, above which no remarkable influences being perceived. The lost or weakened action by heating or by the treatment of alkali is no more recovered. In the medium removed oxygen, the power is also reduced but recovered in this case by supply of the air. Only in the anhydrous alcohol the formation of aldehyde is quite restricted. The period of steeping is worthy of care and once practically aldehyde produced in 15% of alcohol solution reached 0.57% after 2 years.

The origin of oxidation is hardly ever separated from the timber with several solvents——water, alcohol, ether, weak acid solution, toluene-water, chloroform-water, petroleum-ether, 50% of glycerine and acetone——and the residue is always active. The oxidizing power is not due to the action of substances like aldehyde or ketone(2), because for those the sunlight is indispensable while in the timber oxidation takes place smoothly even in the cold, dark place. Moreover the action is not the conduct of either terpene(3), oxidase or peroxidase, for the resin of pine-tree or Cryptomeria, terpene oil, flesh potato and wheat-bran have all almost no effect upon the alcohol solution although the reaction of peroxide for KI solution or those of the oxidizing enzymes for guajac tincture etc. were evidently shown. Thus one of the oxidizing enzymes ' dehydrase '(4) or in a wider sense some oxidizing catalysts may be appointed as a main cause of the oxidation. Lately T. Higashi(5) discovered the action in the rectified Cryptomeria oil, a sesquiterpene and designated it as a main cause, This explanation is very convenient for many

Coniferaes and perhaps it is so in some cases but embarrassed in the cases of persimmon, prunus etc. Moreover we see that the action of terpenes is not so strong as the timbers.

## Experimental.

In every case ca. 15%$^{\times}$ alcohol solution with 3.5 g. of chips of timber for each 100 c.c. of the solution enclosed in a bottle was used and aldehyde was estimated after 14 days at ordinary temperature.

### 1. Aldehyde formation with several timbers continued.

Pine 0.02804 g. Chamaecyparis obtusa 0.01300 g. Crape-myrtle 0.00228 g. Chestnut 0.00170 g. Plum 0.00205 g. Persimmon 0.00448 ; 0.00887 g. Apple 0.00124 g. Mulberry 0.00151 g. Fatsia japonica 0.00232 g. Fig 0.00097. Maple 0.00206 g. Oak 0.00159 g. Cercidiphyllum japonicum 0.00136 g. Zelkova acuminata 0.00182 g. Ebony 0.00410 g. Olegon-pine 0.02241 g. White cedar 0.00354 g. Abies firma 0.00164 g. Celtis sinensis 0.00094 g. Prunus communis 0.00968 g. Cherry 0.01091.

Pomegramate, Gingko, Sultan's parasol, Camellia, Thea, Cape-jasmine, Rose-wood, Western red cedar, White beech, Mahogany, Red cedar, Wall-nut, Lignumvitae, Serayah, Cork, Liquorice, Pasania cuspidata, Willow, Elaegmus pungens had no effect.

### 2. The acition of the different positions. (Following data are all with Cryptomeria.)

(i) Bark (fresh), Twig (fresh), Inactive ; Leaf (fresh) 0.00087 g. Leaf (dry) 0.00391.

| | | I | II | III | IV | V | VI |
|---|---|---|---|---|---|---|---|
| | | g. | g. | g. | g. | g. | g. |
| (ii) | heart-wood | 0.02894 | 0.02200 | 0.01158 | 0.00873 | 0.01559 | 0.00726 |
| | middle part | —— | —— | —— | 0.01513 | —— | 0.02037 |
| | sap-wood | 0.00145 | 0.01506 | 0.00232 | 0.00495 | 0.02604 | 0.01164 |

(iii) With timbers for storage pail or cask.

The middle part between heart-and sap-wood is said to be the most suitable for the demand of saké-brewing and as a rule white-wood (sap) is used outside.

| | A | | B | | C | |
|---|---|---|---|---|---|---|
| | red wood side | white wood side | red | white | red | white |
| | g. | g. | g. | g. | g. | g. |
| after 14 days | 0.01521 | 0 | 0.01304 | 0.00792 | 0.01400 | 0.01557 |
| after 32 days | 0.02380 | 0.00169 | 0.02546 | 0.02101 | 0.02463 | 0.03574 |
| after 90 days | 0.03976 | 0.00170 | 0.18012 | 0.08914 | 0.08925 | 0.08763 |

### 3. Influence of temperature.

i. The bottle was incubated for 2 weeks.

| 7° | 20° | 26° | 30° | 40° | 50° |
|---|---|---|---|---|---|
| 0.01621 g. | 0.01772 g. | 0.02681 g. | 0.02479 g. | 0.02850 g. | 0.02378 g. |

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× average content of saké.

ii. With the timbers treated in hot water at following temperatures for 30 minutes.

| 60° | 70° | 80° | 90° | 100° | 100°×<br>(5 min.) | 100°×<br>(10 min.) | 100°×<br>(15 min.) |
|---|---|---|---|---|---|---|---|
| 0.00897 g. | 0.00717 g. | 0.00299 g. | 0.00119 g. | 0 | 0.00642 g. | 0.00257 g. | 0.00174 g. |

iii. With timbers treated in the dry state.

| 100° 5 h. | 110° 1 h. | 120° 1 h. | 130° 1 h. | 140° 1 h. | 150° 1 h. |
|---|---|---|---|---|---|
| 0.00996 g. | 0 00648 g. | 0.00594 g. | 0.00324 g. | 0.00270 g. | 0 |

4. In anhydrous alcohol. Aldehyde formed 0.00146%.

5. The effect of the reaction.

| PH 2.2 | 3.0 | 6.0 | 7.0 | 8.4 | 9.8 | $Na_2CO_3$ 1g. | $Na_2CO_3$ 1.5g. |
|---|---|---|---|---|---|---|---|
| 0.01415 g. | 0.01938 g. | 0.01179 g. | 0.01703 g. | 0.01257 g. | 0.00917 g. | 0.00419 g. | 0.00367 g. |

6. Removal of oxygen with alkaline pyrogallol solution.

| | A | B |
|---|---|---|
| Removal (after 14 days) | 0.00669 g. | 0.00690 g. |
| Supply (after 53 days) | 0.02275 g. | 0.01600 g. |

7. With the extracts and residues of timbers.

| Solvent | Ether | Water | Alcohol (73%) | Pet.-ether |
|---|---|---|---|---|
| Period of extraction | 3 days | 35 days | 35 days | 2 days |
| With the extract | 0.00428 g. | 0.00136 g. | 0.00156 g. | —— |
| With the residue | 0.04203 g. | —— | —— | 0.01469 g. |
| Period of steeping | 32 days | 35 days | 35 days | 14 days |

| 1% HCl | 1% Lactic | $CHCl_3$ 2 c.c. water 81 c.c. | Toluene 1 c.c. water 82 c.c | Glycerine 50% | Acetone, ether, alc. |
|---|---|---|---|---|---|
| 12 days | 12 days | 2 days | 2 days | 2 days | 2 days |
| 0 | 0 | 0 | 0 | 0 | — |
| —— | —— | 0.01335 | 0.01643 | 0.01971 | 0.00478 |
| 14 days | 14 days | 14 days | 14 days | 14 days | 14 days |

8. Comparison of oxidizing action of timber with that of aldehyde or ketone (p-benzo-chinone as a representative).

| | Treatment | Color of sol. | Aldehyde | Remarks |
|---|---|---|---|---|
| (1) | Timber 35 h. under direct sun light | brown | 0.01273 g. | no special increase |
| (2) | Chinone 0.5 g. | black | 0.06299 g. | Riminis react. blue |
| (3) | Chinone 14 days in the dark place | greyish brown | 0.00256 g. | |
| (4) | Camphor 1.5 g. 〃 | Colorless | 0 | |
| (5) | Hydrochinone 0.5 g. 〃 | Yellow | 0 | |
| (6) | Pyrogallol 0.5 g. 〃 | Pale brown | 0 | |

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(1) M. Yamada: Bull. Agri. Chem. Soc., Jap , 4 22—24, (1928).

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(3) Waksman and Davison: Enzymes p. 230 (1926).

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(5) T. Higashi: Bull. Inst. Phys. Chem. Research, 8 97, (1929).

(From the Imperial Brewing Experimental Station, Takinogawa-machi near Tokyo.)

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× 5% alcohol solution.

# ON VITAMINS IN SAKE-CAKE.

## III. ON ERGOSTERIN IN SAKE-CAKE.

By

EIJI TAKAHASHI and H. LIM.

(Received Dec. 15 th., 1929).

The present paper deals with the separation of Ergosterin from Sake-Cake and Koji-fungus (Aspergillus Oryzae).

1 kg. of dried Sake-Cake was extracted with 85~95% alcohol at 80°C. The extract was evaporated and treated with ether. The substance soluble in the later solvent was separated and refined with acetone. 125 g. of oily substance thus obtained was next saponified with alcoholic potash solution and the unsaponifiable part was taken with ether. By evaporation, Sterin-substance which made of needle and plate like crystals was obtained. The melting point showed 137°C.

As it was considered from the result that the separated substance contained the other sterin having higher melting point than phytosterin, the biological test was next executed.

Albino rats was taken, Steenbock's No. 2965 was given as basal diet, the symptom of ricket was diagnosed by lentogen. When the rats were fed for 5 weeks with above diet, they indicated the characteristic pathological change at the end of shinbone. But by feeding the isolated crystals which were formerly irradiated, cured or prevented the disease. 1/500 mg. of the crystal was able for prevention and 1/50 mg. for cure.

The irradiation was done as follows:

The sample was dissolved in Petri-dish with ether, after evaporation was exposed to the light at the distance of 25 cm. for 40 minutes. The lamp was such small type as passing 2 ampere, 90~100 volt.

Further the separation of ergosterin from the Koji-fungus which was cultivated in the solution free from organic substance but sugar, was tried and the animal experiment was also done. By the same method as above 1.25 g. needle crystals were obtained from 500 g. sample. Its melting point was 163~165°C. $[\alpha]_D^{18} = -126°$, these agree well with ergosterin.

The analytical result was as follows:

32.36 mg. sample gave $CO_2$ 100.33 mg. $H_2O$ 31.89 mg.

| | C | H |
|---|---|---|
| Calculated for $C_{27}H_{42}O$ | 84.75% | 11.07% |
| Found | 84.55% | 10.95% |

The experiment with rats showed 1/5000 mg. prevented the disease and 1/1000 mg. cured it after 3 weeks.

# UEBER DIE OBERFLÄCHENAKTIVITÄT UND DIE ADSORBIERBARKEIT VON AMINOSÄUREN.

## I. MITTEILUNG.

Von

TAKEO ITO.

(*Aus dem Agrikulturchemischen Laboratorium in Landwirtschaftlichen Hochschule, Morioka*)

(Angenommen, Jan. 12, 1930).

In den Untersuchungen über die Adsorption von Aminosäuren und Polypeptiden durch Tierkohle haben E. Abderhalden und A. Fodor(1) gezeigt unter anderem, dass sie in mitteleren Konzentrationen nach der gewöhnlichen Adsorptionsisotherme adsorbiert werden, während bei kleinen Konzentrationen das Henrysche Gesetz gilt. Sie nahmen, um die von ihnen beobachteten Tatsachen erklären zu können, an, dass die Adsorption von genannten Stoffen an Tierkohle kein rein physikalischer Vorgang, vielmehr chemischen Kräften zuzuschreiben sei.

Die vorliegende Untersuchung wurde unternommen, um zu pfrüfen, ob sich die wohlbekannte Beziehung zwischen der Oberflächenaktivität und der Adsorbierbarkeit von oberflächenaktiven Stoffen, wie z.B. Fettsäuren, auch bei Aminosäuren erkennen lässt.

( 1 ) Die zur Untersuchung herangezogenen Aminosäuren wurden teils (Glykokoll, d–Alanin, l–Leucin und d–Glutaminsäure) nach Estermethode aus den sie reichlich enthaltenden Eiweisskörpern, teils (dl–$\alpha$–Aminobuttersäure und dl–Valin) synthetisch aus den entsprechenden Fettsäuren hergestellt. dl–Asparaginsäure war ein Präparat von Merck („für wissenschaftliche Zwecke"). Ihre Reinheit wurde analytisch bzw. physikalisch-chemisch bestätigt.

( 2 ) Die Oberflächenspannung der wässerigen Aminosäurelösungen bei verschiedenen Konzentrationen wurde bei 18 ± 0,5°C mit Hilfe von Tropfenmethode gemessen, indem man ein Stalagmometer nach Traube benutzte.
Die Ergebnisse finden sich in Fig. 1 und 2.

In oben stehenden Figuren ist die relative Oberflachenspannung $\sigma$, bezogen auf die des Wassers, die gleich 1 gesetzt wird, als Ordinate und die Konzentration c in Mol im Liter als Abszisse zugeordnet. Man ersieht aus Fig. 1, dass die Aminomonocarbonsäuren merklich weniger oberflächenaktiv sind als die entsprechenden Fettsäuren (vgl. hierzu Freudlich, Kapillarchemie, 3. Aufl. (1923)).

Glykokoll und Alanin erhöhen sogar die Oberflächenspannung des Wassers. Trotzdem findet sich die Regelmässigkeit auch hier statt, mit der die Oberflächenspannung mit dem Ansteigen in der homologen Reihe zunimmt. Wie aus Fig. 2 ersichtlich, sind Asparaginsäure und Glutaminsäure oberflächeninaktiv.

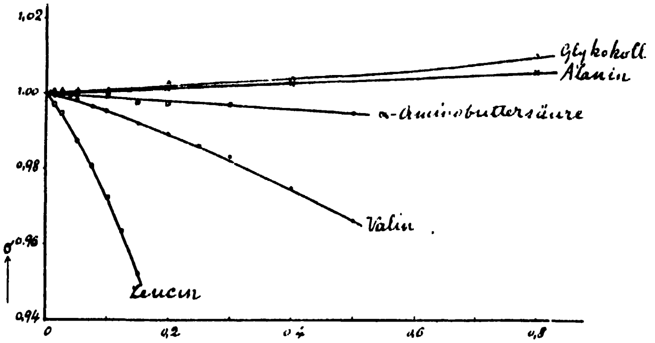

Fig 1
σ-c-Kurven von wäßrigen
α-Aminomonocarbonsaurelösungen.

(3) Für Adsorptionsversuche verwendete ich Knochenkohle (Merck, Carbo animalis pur. sicc.), die bei 100 ~110°C bis zum konstanten Gewichte getrocknet worden war.

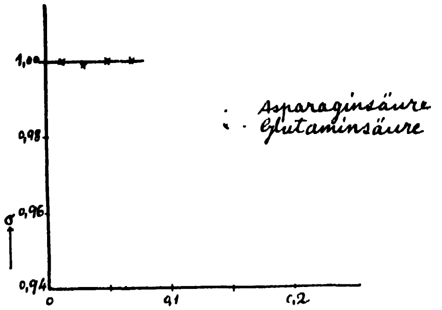

Fig. 2
σ-c-Kurven von wäßrigen
α-Aminodicarbonsäurelösungen.

Ca. 1,25g. Kohle wurden in einen kleinen mit eingeschliffenem Glasstopfen verschliessbaren Erlenmeyerkolben genau eingewogen und mit 25 ccm Aminosäurelösung, die vorher auf 18°C gebracht worden war, zusammengebracht. Nach 20 Minuten Schütteln im Thermostat bei 18±0,1°C wurde die Kohle abfiltriert, wobei die ersten ca. 5 ccm. Filtrat weggeworfen wurden. Der Aminosäuregehalt der Lösungen nach wie vor der Adsorption wurde nach Van Slyke bestimmt und aus der Differenz zwischen beiden Bestimmungen liess sich die adsorbierte Menge berechnen.

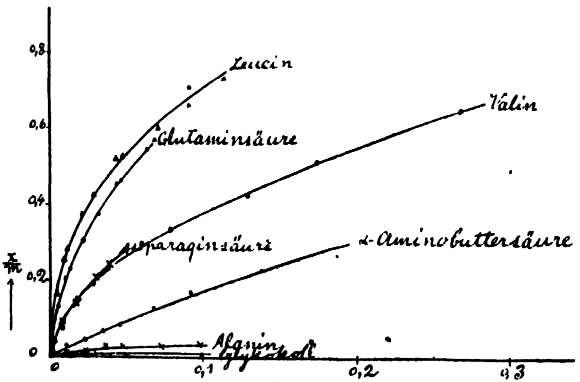

Fig. 3.
Adsorptionsisothermen von
wäßrigen Aminosäurelösungen

Die Resultate sind in Fig. 3 graphisch dargestellt, wobei als Ordinaten $m/x$ die adsorbierten Aminosäuremengen in Millimol pro g.

Kohle, als Abszissen c die Endkonzentrationen in Mol im Liter aufgetragen sind.

Es ist aus oben stehender Figur zu ersehen, dass die Aminosäuren durch Tierkohle geringer adsorbiert werden als die entsprechenden Fettsäuren. Vergleicht man Fig. 1 mit Fig. 3, so erkennt man einen deutlichen Parallelismus zwischen der Oberflächenaktivität und der Adsorbierbarkeit von Aminomonocarbonsäuren.

Zur näheren Orientierung sind in Tabelle I die aus Fig. 1 graphisch ermittelten $m/x$-Werte und die relativen Oberflächenspannungswerte $\sigma$ ($\sigma_{H_2O}=1$) zusammengestellt, beide bezogen auf eine Gleichgewichtskonzentration von 0,1 Mol im Liter.

### TABELLE I.

| Aminosäure | Relative Oberflächenspannung $\sigma$ bei $c=0,1$ Mol i. L. | Adsorbierte Menge $m/x$ in Millimol pro g Kohle bei $c=0,1$ Mol i. L. |
|---|---|---|
| Glykokoll | 1,001 | 0,01 |
| Alanin | 1,000 | 0,035 |
| α-Aminobuttersäure | 0,9989 | 0,17 |
| Valin | 0,995 | 0,39 |
| Leucin | 0,9722 | 0,71 |

Hieraus ersieht man, dass die in der homologen Reihe höher stehenden Aminosäuren merklich oberflächenaktiv und dementsprechend ziemlich gut adsorbierbar sind, und zwar in der Reihenfolge.

α-Aminobuttersäure $<$ Valin $<$ Leucin.

Bemerkenswert ist, dass Glykokoll und Alanin, die bei der in Betracht kommenden Konzentration die Oberflächenspannung des Wassers gar nicht erniedrigen, eher ein wenig erhöhen, durch Kohle aufgenommen werden, wenn auch in geringem Masse.

Asparaginsäure und Glutaminsäure werden, wie aus Fig. 3 ersichtlich, an Kohle merklich adsorbiert, obwohl sie, wie schon erwähnt, oberflächeninaktiv sind. Indessen ist ähnliche Erscheinung auch bei anderen Stoffen, wie z.B. Bernsteinsäure, einigen Zuckerarten usw., bekannt (vgl. Freundlich, loc. cit., S. 268).

Tabelle 2 und 3 enthalten die Ergebnisse der Adsorptionsversuche, wobei für 25 ccm. Aminosäurelösung 1,25 bzw. 1,24/4 g. Kohle angewandt wurden.

### TABELLE II.

Adsorption von Leucin an Knochenkohle.

$$x/m = 1{,}87\,C^{0{,}422}$$

| Kohlenmenge in g. | Anfangskonzentration in Mol i. L. | Endkonzentration c in Mol i. L. | Adsorbierte Menge in Millimol pro g. Kohle | |
|---|---|---|---|---|
| | | | $x/m$ (beob.) | $x/m$ (berech.) |
| 1,2524 | 0,00625 | 0,0015 | 0,095 | 0,1203 |

| | | | | |
|---|---|---|---|---|
| 0,3131 | 〃 | 0,0042 | 0,160 | 0,1858 |
| 1,2594 | 0,0125 | 0,0042 | 0,164 | 0,1858 |
| 0,3143 | 〃 | 0,0093 | 0,257 | 0,2597 |
| 1,2296 | 0,025 | 0,0112 | 0,281 | 0,2809 |
| 0,3094 | 〃 | 0,0203 | 0,376 | 0,3611 |
| 1,2570 | 0,05 | 0,0287 | 0,423 | 0,4149 |
| 0,3145 | 〃 | 0,0435 | 0,519 | 0,4981 |
| 1,2560 | 0,075 | 0,0484 | 0,529 | 0,5210 |
| 0,3127 | 〃 | 0,0678 | 0,571 | 0,6007 |
| 1,2510 | 0,1 | 0,0699 | 0,600 | 0,6084 |
| 0,3162 | 〃 | 0,0914 | 0,708 | 0,6813 |
| 1,2530 | 0,125 | 0,0918 | 0,661 | 0,6829 |
| 1,2548 | 0,15 | 0,1132 | 0,733 | 0,7457 |

## TABELLE III.

Adsoption von Glutaminsäure an Knochenkohle.

$$x/m = 2{,}53^{\circ}C^{0.554}$$

| Kohlenmenge in g. | Anfangskonzentration in Mol i. L. | Endkonzentration c in Mol i. L. | Adsorbierte Menge in Millimol pro g Kohle. | |
|---|---|---|---|---|
| | | | $x/m$ (beob.) | $x/m$ (berech.) |
| 1,2536 | 0,00625 | 0,0026 | 0,072 | 0,0935 |
| 0,3112 | 〃 | 0,0040 | 0,098 | 0,1187 |
| 1,2510 | 0,0125 | 0,0060 | 0,129 | 0,1469 |
| 0,3109 | 〃 | 0,0100 | 0,204 | 0,1973 |
| 1,2562 | 0,025 | 0,0134 | 0,230 | 0,2320 |
| 0,3152 | 〃 | 0,0213 | 0,302 | 0,2999 |
| 1,2583 | 0,05 | 0,0313 | 0,372 | 0,3712 |
| 0,3124 | 〃 | 0,0444 | 0,455 | 0,4506 |
| 1,2537 | 0,07 | 0,0464 | 0,470 | 0,4617 |
| 0,3103 | 〃 | 0,0632 | 0,544 | 0,5477 |

In oben stehenden beiden Tabellen sind neben der experimentell ermittelten Grösse $x/m$ die nach der an der Spitze der betreffenden Tabelle angeführten Formel berechneten $x/m$-Werte angegeben. Die experimentell ermittelten und die berechneten Werte sind sehr nahe. Mit anderen Worten: Bei Leucin bzw. Glutaminsäure–und ähnliches dürfte auch für die anderen Aminosäuren gelten–lässt sich die Adsorption, unabhängig von der angewandten Kohlenmenge, je durch eine Adsorptionsisotherme wiedergebeı

## LITERATURE.

(1) Fermentforschung: 2, 74 (1917); 2, 151 (1918).
Kolloid Zeitschr: 27, 49 (1920).

# STUDIES ON BACILLUS THERMOFIBRINCOLUS N. SP. III.

## PRODUCTION OF VOLATILE ACID AND ALCOHOL.

By

ARAO ITANO and SATIYO ARAKAWA

(Received Jan. 20 th., 1930).

The results of further investigation on B. thermofibrincolus n. sp. as to the production of volatile acid and alcohol together with the quantitative determination of cellulose fermented, are reported below:

( 1 ) In course of cultivation of the organism in a test tube at 65°C., 45.5% evaporation takes place in a week. But it can be prevented by placing an inoculated tube in another larger tube with a cork stopper which has small holles.

( 2 ) More cellulose is fermented in a test tube than that in a 300 c.c. flask although more volatile acid and alcohol are produced in the latter. At 65°C, the organism ferments 60~88% cellulose supplied at the beginning, in 3~7 days, and produce 24~33% volatile acid, as acetic acid and 3~12% alcohol, as etheyl alcohol in proportion to the quantity of cellulose fermented.

( 3 ) The optimum temperature for the organism is 65°C., and at 50°C. ferments only 63% cellulose as to that fermented at 65°C. But the amount of volatile acid and alcohol produced is rather large at 50°C., comparing to that at 65°C. This may be due to the facts that some of the products are lost at the higher temperature and also used up in the metabolic process.

( 4 ) As to the influence of oxygen, less quantity of cellulose is fermented under anaerobic condition (Buchner's method) than that under aerobic condition. Although a similar amount of volatile acid is produced in both cases, more than the double amount of alcohol is produced under the anaerobic condition.

( 5 ) The influence of different concentration (0.0~2.0%) of peptone was determined and found 0.5% is the optimum, and the less than the optimum amount gives better fermentation than that at higher concentration. Even without an addition of peptone, fairly good fermentation takes place. The quantity of volatile acid produced parallels the quantity of peptone given.

( 6 ) Different amount of cellulose has different influence; 0.75~2.20% inclusive is the best for both cellulose fermentation and volatile acid production but for alcoholic production, the larger amount of cellulose is better.

( 7 ) As to the influence of nitrogen source, the following relations were found:

For cellulose fermentation;

peptone > egg albumin > casein > ammoniun sulfate > meat extract.

For volatile acid production;

meat extract > egg albumin > peptone > casein > ammonium sulfate.

For alcoholic production;

meat extract > peptone > egg albumin > casein > ammonium sulfate.

Thus the organism seems to require the organic nitrogen for both production of volatile acid and alcohol.

( 8 ) In the medium which contains $CaCO_3$, 64% cellulose fermented on the third day while only 32%, without $CaCO_3$: on the seventh day, 88% fermented in the former and 46% in the latter. The production of both the volatile acid and alcohol is greatest on the third day. Such marked difference of fermentation seems to be due to the accumulation of organic acid which interferes with the process where no $CaCO_3$ is present. Again on this basis, it is possible to follow the physiological activity of the organism by determining the pH values.

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# STUDIES ON BACILLUS THERMOFIBRINCOLUS N. SP. VI.

## NITROGEN METABOLISM.

By

Arao Itano and Satiyo Arakawa

(Received Jan, 20 th., 1930).

The action of the organism as to the transformation of amino and ammonia nitrogen in the culture medium with and without an addition of cellulose.

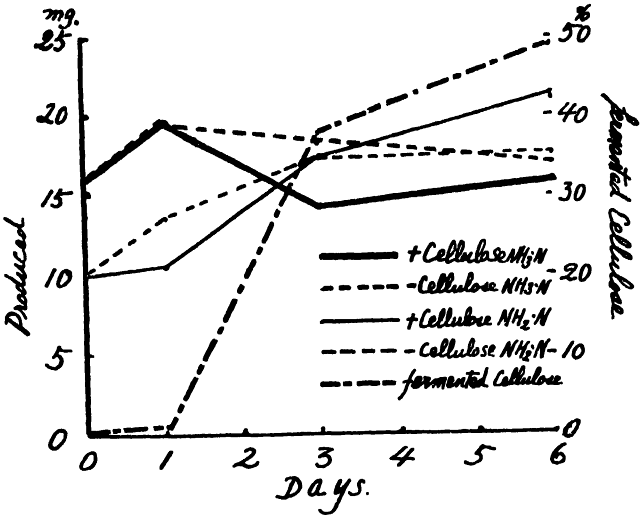
The results are shown in the following figure; Fig. I.

As Fig. I. indicates, in presence of cellulose, the quantity of ammonia nitrogen produced is irregular while the amino nitrogen increased steadily. In case no cellulose is present, both ammonia and amino nitrogen decreased after certain days. Consequently in presence of cellulose, the quantity of amino acid can serve as an approximate index of proteolysis, and it seems to

indicate that the organism contains the proteolytic enzyme. This confirms the experimental results obtained by De Bord.

Fig. I.

Nitrogen Metabolism.



# UEBER DAS VORKOMMEN VON SCHWEFLIGER SAEURE IN VERGOERENER FLUESSIGKEIT.

Von

Mosuke Matsuyama und Hiroichi Nakamura

*(Aus dem Laboratorium der Union-Brauerei bei Tokyo, Japan.)*

(Angenommen Jan, 25, 1930).

Obgleich wir in der vorhandenen Literatur mehrfach ueber Versuche mit Schwefelwasserstoff in vergorener Fluessigkeit gelesen haben, haben wir bisher nichts gefunden ueber die schweflige Saeure, die auch stets in kleinen Mengen in vergorener Fluessigkeit, z.B. in Bier und Wein, enthalten ist. Wir haben Untersuchungen auf diesem Gebiet angestellt und teilen die Resultate in folgendem mit:

Nach einigen Vorversuchen wiesen wir das Vorhandensein von schwefliger Saeure wie folgt nach:

Zu 200 ccm der Probe fuegt man 10 ccm von 25%iger Phosphorsaeureloesung hinzu und destilliert unter Durchleitung von durch Kaliumpermanganat-Loesung und Wasser gewaschener Kohlensaeure, bis 100 ccm Destillat gewonnen ist. Als Vorlagekolben dient ein Erlenmeyer'scher Kolben, welcher 20 ccm Jod-Jodkalium-Loesung (5 g. I und 7,5 g. KI in 1 ltr. Wasser geloest) enthaelt. Man setzt dem Destillat 10 ccm konzentrierte Salzsaeure hinzu und erhitzt es, bis die Jodfarbe verschwindet. Dann bestimmit man die Schwefelsaeure als Bariumsulfat durch Zusatz von 20 ccm 10%iger Bariumchloridloesung.

Zunaechst wurd die schweflige Saeure im Rohstoff bestimmit. Die Probe wurde mit 200 ccm Wasser und 0,5 g. Sodiumcarbonat 6 Stunden extrahiert, und die Auszuege wurden in der oben angegebenen Weise behandelt. Wir stellen die Resultate in folgender Tabelle zusammen:

| Rohstoffe | Probe (g.) | Schweflige Saeure (g.) (als $BaSO_4$) | Schweflige Saeure in 100 g. Substanz |
|---|---|---|---|
| Malz I (direkt gedarrt) | 30 | 0,0018 | 0,0060 |
| Malz II (indirekt gedarrt) | 30 | 0,0007 | 0,0023 |
| Hopfen (Saazer I) | 10 | 0,0071 | 0,0710 |
| Hopfen (Saazer II) | 10 | 0,0162 | 0,1620 |
| Hopfen (amerikanisch) | 10 | 0,0428 | 0,4280 |
| Reis | 30 | 0,0010 | 0,0033 |

Wir haben sodann mit 270 g. Malz II, 80 g. Reis, 6 g. Hopfen (Saazer II) und Wasser 2 ltr. den Wuerze gemaischt und die schweflige Saeure derselben bestimmit. Nach einer auf Grund der gebrauchten Rohstoffe angestellten Berechung haetten 100 ccm Wuerze 0.0009 g. schweflige Saeure (als $BaSO_4$) enthalten muessen; wir fanden jedoch, dass die Wuerze nur geringe (0,0001 in 100 ccm) und nicht genuegend bestimmbare Mengen enthielt. 700 g. Malzschrot wurden mit 3,5 ltr. Wasser gemaischt, mit 10,5 g. Saazer Hopfen gehopft, und nach einstuendigem Kochen wurde die Wuerze durch Zusatz von Wasser geeicht, sodass sie einem Extrakt von 12,5° Balling entsprach. Zum Maischen wurden verschiedene Arten Wasser gebraucht und verschiedene Sorten Wuerze gewonnen. 500 ccm der Wuerze wurden in Kolben verteilt, sterilisiert und mit Bierhefe vergoren in einem Zimmer mit einer Temperatur von 10∼12°C (Hierfuer wurden zwei verschiedene Staemme von untergaeriger Hefe unserer Brauerei benutzt, welche wir mit A und B bezeichnen). Vor und nach der Gaerung wurde die schweflige Saeure bestimmt. Die Resultate sind aus folgenden Tabellen ersichtlich.

Wuerze mit destilliertem Wasser:

|  | Vergaerungstage | Probe (ccm.) | $SO_2$ (g.) (als $BaSO_4$) | ″ in 1 ltr. | ″ Zunahme |
|---|---|---|---|---|---|
| Wuerze | 0 | 200 | 0 | 0 |  |

| | | | | | |
|---|---|---|---|---|---|
| Vergoren mit Hefe A | 9 | 200 | 0,0012 | 0,0060 | 0,0060 |
| 〃 〃 〃 B | 9 | 200 | 0,0012 | 0,0060 | 0,0060 |

Wuerze mit Brunnenwasser:

| | | | | | |
|---|---|---|---|---|---|
| Wuerze | 0 | 200 | 0,0002 | 0,0010 | |
| Vergoren mit Hefe A | 9 | 200 | 0,0012 | 0,0060 | 0,0050 |
| 〃 〃 〃 B | 9 | 200 | 0,0010 | 0,0050 | 0,0040 |

Wuerze mit Magnesiumsulfat-Wasser (0,28 g. $MgSO_4$ in 1 ltr.):

| | | | | | |
|---|---|---|---|---|---|
| Wuerze | 0 | 200 | 0,0004 | 0,0020 | |
| Vergoren mit Hefe A | 9 | 200 | 0,0012 | 0,0060 | 0,0040 |
| 〃 〃 〃 B | 9 | 200 | 0,0022 | 0,0110 | 0,0090 |

Wuerze mit Gips-Wasser (0,28 g. $CaSO_4 \cdot 2H_2O$ in 1 ltr.):

Wuerze mit Gips-Wasser (0,34 g. $CaSO_4 \cdot 2H_2O$ in 1 ltr.):

| | | | | | |
|---|---|---|---|---|---|
| Wuerze | 0 | 200 | 0,0002 | 0,0010 | |
| Vergoren mit Hefe A | 9 | 200 | 0,0012 | 0,0060 | 0,0050 |
| 〃 〃 〃 B | 9 | 200 | 0,0056 | 0,0280 | 0,0270 |

Wuerze mit Gips-Wasser (0,25 g. $CaSO_4 \cdot 2H_2O$ in 1 ltr.):

| | Vergaerungstage | Scheinbarer Extrakt (Balling) | Probe (ccm) | $SO_2$ (g) (als $BaSO_4$) | 〃 in 1 ltr. | 〃 Zunahme |
|---|---|---|---|---|---|---|
| Wuerze | 0 | 12,5 | 200 | 0,0002 | 0,0010 | |
| Vergoren mit Hefe B | 3 | 11,0 | 200 | 0,0008 | 0,0040 | 0,0030 |
| | 9 | 6,5 | 200 | 0,0036 | 0,0180 | 0,0170 |

## Zusammenfassung.

(1) Die gehopfte und gekochte Wuerze aus Malz und Reis hat einen geringen Gehalt an schwefliger Saeure, welcher sich vergroessert durch Gaerung mit Bierhefe.

(2) Die Bildung von schwefliger Saeure bei alkoholischer Gaerung steht in naher Beziehung zu den Hefestaemmen und der Art und Menge des Sulfats in der Fluessigkeit.

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# UEBER DIE VERTEILUNG VON PHOSPHORSAEURE IN GERSTE UND MALZ.

Von

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*(Aus dem Laboratorium der Union-Brauerei bei Tokyo, Japan.)*

(Angenommen Jan. 25, 1930).

Ueber die in Gerste und Malz vorkommende Phosphorsaeure liegen bereits aus anderen Laboratorien viele Untersuchungen vor, deren Resultate jedoch in einigen Punkted voneinander abweichen. Wir haben ebenfalls Untersuchungen ueber die Verteilung von Phosphorsaeure in Gerste und Malz vorgenommen. Als Untersuchungsmaterial diente in der Tochigi-Gegend im Jahre 1928 geerntete "Goldenmelon" und von derselben Gerste gekeimtes Malz. 20 g. von jeder Probe wurde zunaechst mit Aether und nacheinander mit Alkohol, 0.2% Salzsaeure und 10% Ammoniak extrahiert und die Phosphorsaeure—die Gesamtmenge und noetigenfalls auch die Menge von organischer und anorganischer Form—in jedem Auszug bestimmt. Weiterhin wurden 20 g. Proben mit 0.2%iger Salzsaeure extrahiert, und mit dem Auszug wurde die Phytin–Phosphorsaeure durch Faellung mit Kupferracetat–Loesung und weitere Behandlung mit Schwefelwasserstoff, Salzsaeure und Baryta bestimmt. Es wurde auch das Vorhandensein von Phosphoproteid–Phosphor, welcher durch Pepsin–Salzsaeureloesung (1% Pepsin in 0.2% HCl) ausgezogen wurde, bestimmt. Die Bestimmung der Phosphorsaeure wurde vorgenommen nach dem Verfahren von Lorenz, und die anorganishe Phosphorsaeure wurde bestimmt nach der Methode Schulze–Castro. Bei unseren Untersuchungen kamen wir zu folgenden Resultaten, wobei die Einzelergebnisse im Verhaeltniss zu $P_2O_5=100$ umgerechnet sind.

| | Gerte | | Malz | |
|---|---|---|---|---|
| | in Trockensubst. | % von Gesamt-$P_2O_5$ | in Trockensubst. | % von Gesamt-$P_2O_5$ |
| Gesamt-Phosphorsaeure | 0,8570 | 100 | 0,8342 | 100 |
| Phosphatid–$P_2O_5$ | 0,0987 | 11,52 | 0,0805 | 9,65 |
| Anorganische–$P_2O_5$ | 0,1778 | 20,75 | 0,2452 | 29,39 |
| Phytin–$P_2O_5$ | 0,0450 | 5,25 | 0,0408 | 4,89 |
| Nuclein–$P_2O_5$ | 0,4915 | 57,35 | 0,4570 | 54,80 |
| Phosphoproteid und unbestimmte–$P_2O_5$ | 0,0440 | 5,13 | 0,0107 | 1,27 |
| | **0,8570** | **100** | **0,8342** | **100** |

In der Tabelle zeigt die Phosphatid-$P_2O_5$ die Summe der in Aether und Alkohol loeslichen Phosphorsaeure, die anorganische die beim sukzessiven Extrahieren in 0.2%iger Salzsäureloesung loesliche, nach dem Verfahren von Schulze-Castro bestimmbare $P_2O_5$, und die Nuclein-$P_2O_5$ die in 10%igem Ammoniak loesliche Phosphorsaeure. Phytin-$P_2O_5$ wurde einzeln in der oben beschriebenen Weise bestimmt. Die Differenz zwischen der Gesamt-$P_2O_5$ (welche mit Asche von 3 g. Proben direkt bestimmt wurde) und der Summe von gegebenen anderen $P_2O_5$ bezeichneten wir als Phosphoproteid–und unbestimmte $P_2O_5$.

Aus unseren Ergebnissen ist ersichtlich, dass der Gehalt an organischen Phosphorsaeuren bei Malz geringer ist als bei Gerste, waehrend es sich mit anorganischen Phosphorsaeuren umgekehrt verhaelt. Hieraus erklaert sich die Wirkung von Phosphatasen waehrend der Keimung.

# EFFECT OF STANNOUS CHLORIDE ON THE ACID HYDROLYSIS OF PROTEIN AS A PREVENTIVE AGENT FOR THE FORMATION OF "MELANIN SUBSTANCE".

By

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(Received Jan, 10 th., 1930).

The so-called "humin" or "melanin substance", darkly coloured and of unknown chemical constitution, are formed when proteins are boiled with mineral acid. Though our knowledge about the chemical nature of this substance is yet very limited, we can trace the origin of this substance which consists of some special amino acids, tyrosine, cystine, tryptophane and hexon bases on the one hand, and the carbohydrate accompaning proteins such as glucose or glucosamine on the other.

In the analytical courses of protein hydrolysate, the "melanin substance" makes many disadvantages about the accurate estimation of amino acids, therefore the devices to prevent the formation of "melanin substance" on the acid hydrolysis of protein were made, and Hlawetz and Haberman were the first who employed stannous chloride for this purpose. Some investigators followed this indication, but none payed attention about its applicability to the quantitative analysis of amino acids. Then the above matters were tested:

(1) By application of $SnCl_2$ to the nitrogen distribution analysis of the Van Slyke's method. The effect of $SnCl_2$ on hydrolising casein only and on hydrolising casein with glucose was as in results belows:

| | Casein only (3 g.) | | | | Casein (3 g.) with glucose (1 g.) | | | |
|---|---|---|---|---|---|---|---|---|
| | Usual method | | $SnCl_2$ method | | Usual method | | $SnCl_2$ method | |
| Melanin-N. | 1.40% | 1.34% | 1.43% | 1.38% | 2.07% | 2.20% | 2.73% | 2.84% |
| Amide-N. | 10.80 | 10.12 | 9.35 | 9.44 | 11.25 | 11.48 | 10.54 | 10.71 |
| Diamino-N. | 24.93 | 27.31 | 27.12 | 26.97 | 26.18 | 24.78 | 26.50 | 26.52 |
| Arginine-N. | 7.90 | 7.87 | 8.86 | 8.67 | 8.33 | 8.07 | 8.85 | 8.59 |
| Histidine-N. | 2.99 | 6.29 | 5.43 | 6.29 | 4.67 | 3.81 | 5.08 | 5.66 |
| Cystine-N. | 1.05 | 1.08 | 1.05 | 1.08 | 1.08 | 1.05 | 1.05 | 1.02 |
| Lysine-N. | 12.99 | 12.06 | 11.76 | 11.23 | 12.10 | 11.85 | 11.42 | 11.23 |
| Monoamino-N. | 56.46 | 55.14 | 54.86 | 55.69 | 53.96 | 55.51 | 55.17 | 55.63 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Nonamino-N. | 6.51 | 6.11 | 5.62 | 5.86 | 8.15 | 7.68 | 6.94 | 6.26 |
| Total-N. recovered | **100.10** | **100 02** | **98 38** | **99.34** | **101·61** | **101·65** | **101.88** | **101.96** |

Note: (1) The value of melanin-N. include:

a) N. in the darkly colored insoluble residue.

b) N. adsorbed with Ca $(OH)_2$ or tin sulphides.

c) N. in the phosphotungstate melanin.

(2) At the above method with $SnCl_2$ the filtrate from tin sulphide is completely colourless so the precipitate of phosphotungstates are mostly snow-white.

Judging from the colour of hydrolysate, the melanin formation is greatly reduced when $SnCl_2$ is added, but the apparent high values of melanin nitrogen at the side of $SnCl_2$ method may be attributed to the greater adsorbtion capacity of tin sulphides than that of $Ca(OH)_2$. The each value in the fraction of phosphotungstate precipitates on the method with $SnCl_2$ coinsides closely together in parallel analysis, and they do not alter when casein is hydrolised with the addition of glucose.

(2) The effect by addition of $SnCl_2$ on the formation of "melanin substance" when amino acids and carbohydrates are boiled together with concentrated hydrochloric acid is as follows: the amino acids, containing 0.05 g. of nitrogen, and 0.1 g. of glucose, were boiled with 100 c.c. of 25% HCl for 5 hours with or without the addition of 1 c.c. of 5n. $SnCl_2$ solution.

In general, regarding the "insoluble melanin substances": their colours, appearances, rough amounts and nitrogen contents, are observed, and as to the soluble forms the colours of solution and nitrogen adsorbed with tin sulphides when the metal is removed by $H_2S$ are also noted. But as for the difference of adsorption capacities of SnS as well as $SnS_2$, this nitrogen values do not indicate the amount of soluble forms.

Glycocoll, leucine, glutaminic acid, histidine, arginine and ammonium chloride gave no or little "melanin substances" cotaining nitrogen. On the contrary tyrosine, cystine and tryptophane gave much "melanin substances", but the addition of $SnCl_2$ gave much or less diminishing effect on melanin formation. By increasing the amount of glucose from 0.1 g. to 0.5 g., the added $SnCl_2$ became deficent.

Nextly, several kinds of carbohydrate and HCl were heated with or without the addition of $SnCl_2$. Glucose and fructose behaved likely, both producing black insoluble powdery substances, and slightly brown filtrate on the control experiment, and dark brown tallowy residue and faint yellow filtrate on the addition of $SnCl_2$. Arabinose also behaved almost likely with the formor cases, but a little different in producing the dark brown powdery "insoluble melanin substances" on the addition of $SnCl_2$. It is quite different in the case of furfurol, the addition of $SnCl_2$ had no effect on reducing the amount of insoluble black melanin which was produced immediately after the mixtures were heated. By this phenomena it is indicated that the added $SnCl_2$ makes its effect at the stage of condensation, sugars into the furfurol like-substances. In fact, if $SnCl_2$ is added the quantity of the furfurol produced from arabinose by the method of pentose estimation, diminished to about one half of the normal value. As to glucosamine the melanin formation is completely prevented by $SnCl_2$, and from mannit no melanin is formed at all.

The next experiment is the heating of amino acids: tyrosine or cystine, or tryptophane, with arabinose or glucosamine or fructose. In this case it is interesting that glucosamine is a very weak melanin producer, that is to say, no insoluble melanin is formed in all cases, also, the soluble formes produced from tyrosine or cystine are almost negligible, and only from tryptophane the dark brown solution is made when no $SnCl_2$ is added, but the colour doesn't appear when the reagent is added.

Throughout this experiment the attempt to express the amount of "melanin substances" by the nitrogen values was in vein, because the tin sulphides, on removing the metal, adsorbed not merely the melanin but also other nitrogeneous compounds. By experiments it was found that arginine, histidine, cystine and tryptophane were especially much adsorbed by stannic sulphide ($SnS_2$).

# ON THE CHANGES OF CYANAMIDE IN THE SOIL. PART I.

## A COMPARATIVE STUDY IN DRY-FIELD AND PADDY-FIELD CONDITIONS.

By

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(Received Dec. 14 th., 1929).

Cyanamide, when applied to the paddy-field, has been found to destroy the icterohaemorrhagic spirochaeta in the soil (Tōyama, 1918). The application of commercial cyanamide to the paddy-rice field gives the good results for prevention of Spirochaetosis icterohaemorrhagicae (so-called Weil's disease), as well as the good fertilizing effect on the rice crop. Consequently, the direct use of commercial cyanamide in the paddy-rice field, prior to the transplantation of paddy-rice, is gradually spreading. Furthermore, the use of commercial cyanamide in various crop cultures is recently becoming more common in Japan, because of its relative cheapness as a nitrogenous fertilizer and its weed-killing and soil pests destroying power. Little, however, is known of the transformation of cyanamide in the paddy-field state. The present work was therefore undertaken to compare the changes of cyanamide nitrogen in two different soil conditions, namely dry-field (normal soil) and paddy-field (water-logged soil).

## Methods.

The several modifications and improvements in analytical methods of cyanamide and its transformation products in the soil were attempted by the author. The ammonia nitrogen was determined by the aeration method using Mattew's aerator and a simple absorber (illustrated in original paper). In extracting the soil for the determinations of nitrate, urea, and dicyanodiamide nitrogen the addition of ferric sulphate or copper sulphate solution (ferric chloride solution in the case of urea determination) and then calcium hydroxide powder, to the soil sample, greatly facilitated the filtration and gave clear filtrate. The determination of nitrate nitrogen was made by reduction method, using, usually, Devarda's alloy, and, sometimes, zinc-copper couple or titanium trichloride solution, while the colorimetric method, using phenoldisulphonic acid, was occasionally employed. For the determination of urea nitrogen, the urease method was adopted, treating the soil extract or the soil itself with 5% water extract of Jack-bean flour. For determining cyanamide nitrogen in the soil the author devised a new method which comprises the conversion of cyanamide into urea by treating the soil sample with two times of 2 normal hydrochloric acid at 50~55°C. for 3 hours. After nutralizing with sodium hydroxide the total urea in the filtrate was estimated by the urease method. The urea, which was present in the soil previous to the treatment with hydrochloric acid, having been determined separately, as described before, the cyanamide nitrogen was obtained by difference. As the sodium chloride, formed in the solution, has some depressing effect on the action of the urease, the urea decomposing power of Jack-bean flour extract in 5% solution of sodium chloride should be tested previously. This new method is also applicable to the analysis of commercial cyanamide, giving fairly satisfactory agreement with the volumetric silver cyanamide method and gravimetric dixanthyl urea method of Fosse. Lastly, the dicyanodiamide nitrogen in the soil, together with cyanamide nitrogen, was estimated by the method of Brioux, using the soil extract. The cyanamide nitrogen being determined separately by the author's method, the dicyanodiamide nitrogen will be obtained by difference.

The investigations were carried out with three soils of different types, viz, a fine sandy loam, a fine sandy loam rich in humus, and a fine clayey loam. Their mechanical and chemical analyses were thoroughly made, and some of their physical and chemical properties were also examined. The investigations on the changes of cyanamide and its transformation products in the soil were all conducted in bottle experiment, using half air dried fine soil passed through a 2 mm. sieve. The soils were weighed into 100 or 150 gms. lots of dry soil, allowance having been made for the moisture content of the soil. In order to approximate to the actual field practice, the use of higher doses of nitrogen compounds (which were to be tested) was avoided, but usually 5~25 mgs. of N was given per 100 gms. of dry soil, with the exception of the experiment for dicyanodiamide formation from cyanamide, where application of 50~100 mgs. cyanamide N was made. A series of lots receiving no nitrogenous compound served as control in most cases of experiments. Commercial cyanamide was usually sprinkled on to each lot of weighed soil placed on a sheet of paper, and the each was mixed and filled into wide-mouthed bottles. The other nitrogen compounds, viz. ammonium sulphate, urea, and dicyanodiamide, were always added, in aqueous solution, directly into bottles containing the weighed soil. The moisture content of soil was brought up to 1/2 and 1/4 of water capacity of the soil, in the "dry-field condition" and to the saturation point, that is, more than 100% of water capacity, in the "paddy-field condition". The bottles were then plugged with purified cotton-wool, and kept at temperatures varying 8~35°C., each experimental temperature having been recorded continuously by selfrecording thermometer. The water lost by evaporation was replaced at intervals of 2~14 days. A fresh bottle was used for each determination at the end of various periods.

## Experimental results.

### (1) Decomposition of cyanamide in the soil (with 3 tables in original paper.)

The three experiments, *effect of soil types, influence of temperature*, and *effect of method of applying the cyanamide*, were made, dry-field and paddy-field conditions being paralleled in all experiments. The effect of soil types and the influence of temperature upon the ammonia and urea formation from cyanamide were almost same in both soil conditions. The rate of ammonia production from cyanamide was somewhat slower in paddy-field condition than in dry-field condition; and in the former condition the decomposition has stopped at ammonia formation stage, no nitrate having been formed. The effect of perfect and imperfect mixing of cyanamide with soil were distinctly different in both soil conditions, showing smaller effect in paddy-field condition than in dry-field condition. In the dry-field condition, the method of applying the cyanamide affected greatly the decomposition of cyanamide. Particularly, when the moisture content of soil was small and the dose of cyanamide was high the influence was marked, the ammonia formation from cyanamide being retarded considerably in the imperfectly mixed one. These results suffice to show the importance of soil water in bringing the cyanamide solution into contact with soil particles in order to transform the cyanamide, in arable soil. And in the actual dry-field practice, especially in the drought season, it is, therefore, desirable to distribute and work up the commercial cyanamide as uniformly as possible in the soil. Nevertheless, in the paddy-field practice the thorough mixing of cyanamide with soil might not be so important.

### (2) Decomposition of urea in dry-field and paddy-field conditions (with 1 table in original paper).

Ammonification of urea in paddy-field condition was somewhat slower than in dry-field condition. But the conversion of urea into ammonia in both soil condition took place fairly rapidly; even at a relatively lower temperature (about 10°C.) 5 mgs. and 10 mgs. urea nitrogen per 100 mgs. dry soil were almost completely ammonified within 5~7 days and 7~14 days respectively. And after these periods the nitrification had begun in the dry-field condition, but in the paddy-field condition the decomposition had stopped at ammonia formation stage, as would be presumed.

### (3) Dicyanodiamide formation from cyanamide in the soil (with 2 tables in original paper).

The two experiments, *effect of soil types* and *effect of doses of cyanamide*, were conducted. The effect of soil types upon dicyanodiamide formation from cyanamide was marked. With the fine sandy loam, the most inactive of the three soils used for the experiment the disappearance of cyanamide was slow, and more than 40% and 25% of added cyanamide remained unchanged at the end of 2 weeks and 5 weeks respectively, 50 mgs. cyanamide nitrogen per 100 gms. dry soil being added. And the increasing accumulation of dicyanodiamide was noticed, 27% of added nitrogen being transformed into dicyanodiamide at the end of 80 days. Whilst with the fine sandy loam rich in humus and with the fine clayey loam the disappearance of cyanamide was relatively rapid: the large parts of cyanamide disappeared within 2 weeks and 3 weeks respectively. And only small parts of cyanamide were transformed into dicyanodiamide, in both soils.

The dicyanodiamide formation from cyanamide depends largely on the quantity of cyanamide applied. Where the addition of 50 mgs. cyanamide nitrogen per 100 gms. dry soil was made the large parts of cyanamide were ammonified within 5 weeks at 20±5°C. in both soil conditions, and the formation of dicyanodiamide was very small, the fine clayey loam having been used as experimental soil. Whilst where the 100 mgs. cyanamide nitrogen was added to the same soil only three-fourth of added nitrogen was transformed into ammonia within the same period, and the remaining one-fourth was transformed into dicyanodiamide.

From above results, it is clear that the attempt of favourable conversion of cyanamide by mixing with ordinary soils previous to the application to the arable land would be vague and attend some dangers. For, as even with the active clayey loam, less than 2000 parts soil per 1 part cyanamide nitrogen, or about 400 parts soil per 1 part commercial cyanamide, dose not lead beneficially the decomposition of cyanamide without the formation of dicyanodiamide. But as a fertilizer for paddy-rice the case is different, for the dicyanodiamide exerts no harmful influence upon paddy-rice, as will be described later.

### (4) The effect of dicyanodiamide upon the decomposition of cyanamide (with 2 tables in original paper).

Dicyanodiamide, when added at the rate of 0.5～5.0 mgs. N per 100 gms. dry soil, matorially retarded or entirely stopped the nitrification, but did not interfered with the ammonia formation from cyanamide. These results agree with those of other authorities.

### (5) Changes of dicyanodiamide in dry-field and paddy-field conditions, and the manurial value of dicyanodiamide for paddy-rice (with 3 tables and 1 figure in original paper).

These experiments were made with chemically pure dicyanodiamide prepared by the author from commercial cyanamide, showing 66.7% nitrogen by analysis and having good purity when determined as nickel guanyl urea.

The difference in rate of decompotion of dicyanodiamide between dry-field and paddy-field conditions was marked, the comparison being made by determining the produced ammonia and the remaining dicyanodiamide in the soil. In the dry-field condition the ammonification of dicyanodiamide took place slowly, and the influence of temperature was very slight. Whilst in the water-logged paddy-field soil condition the ammonia production from dicyanodiamide at relatively higher temperature (30±5°C.) proceeded fairly rapidly although at lower temperature (8～16°C.) its ammonification and diminution took place much less rapidly.

These results show an intersting fact indicating that the exclusive fertilizing value of dicyanodiamide on paddy-rice may be due to its peculiar nature of decomposition in the soil. For in summer time——the season of rice culture and the prevailing temperature of paddy-field soil is then fairy high——the

| | Time elapsed in days. | Nitrogen present per 100 gms. dry soil (mgs.) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Dicyanodiamide added 25 mgs. N per 100 gms. dry soil. | | | | Dicyanodiamide added 5 mgs. N per 100 gms. dry soil. | | | |
| | | Dry-field condition | | Paddy-field condition | | Dry-field condition | | Paddy-field condition | |
| | | Ammonia N | $C_2N_4H_4$ N | Ammonia N | $C_2N_4H_4$ N | Ammonia N | $C_2H_4N_4$ N | Ammonia N | $C_2N_4H_4$ N |
| At low temperature (16—8—14°C.) | 0 | 0.4 | 25.0 | 0.4 | 25.0 | 0.4 | 5.0 | 0.4 | 5.0 |
| | 30 | 0.8 | | 0.8 | | 0.6 | | 0.6 | |
| | 75 | 1.2 | | 1.2 | | 1.0 | | 1.1 | |
| | 120 | 1.2 | | 1.5 | | 1.0 | 4.4 | 1.6 | 4.4 |
| | 150 | 1.3 | 21.9 | 1.8 | 21.9 | 1.2 | 4.0 | 2.0 | 4.0 |
| At high temperature (30±5°C.) | 0 | 0.5 | 25.0 | 0.5 | 25.0 | 0.5 | 5.0 | 0.5 | 5.0 |
| | 25 | 1.4 | 24.3 | 2.0 | 23.0 | 1.4 | 3.3 | 1.4 | 2.6 |
| | 85 | 3.0 | 23.0 | 18.3 | 3.6 | 2.5 | 2.6 | 6.9 | 0 |
| | 135 | 4.0 | 15.1 | 22.3 | 0 | | | | |

ammonification of dicyanodiamide in water-logged paddy-field soil seems to take place moderately to rapidly to meet the demand of rice plant.

It has long been an accepted fact that dicyanodiamide acts harmfully upon the plant growth when applied to the soil. On the other hand the good fertilizing value of dicyanodiamide on paddy-rice was reported by several experimenters in Japan (Aso, 1909; Tazaki and Taguchi, 1920). With the object of obtaining further information as to the fertilizing value of dicyanodiamide on paddy-rice the author carried out pot experiments with 1/200,000 hectare pots, using pure dicyanodiamide described before and ammonium sulphate for comparison. Each pot held 10 kilos equivalent dry soil of fresh fine sandy loam, allowance having been made for the moisture content of the soil. Dicyanodiamide and ammonium sulphate were tested at the three different rates of 1.0, 0.5, and 0.25 mg. N per pot respectively and applied on the day of transplantation of rice, a series of pots without nitrogen serving as control. A set of two pots was in each series and all received 10 gms. superphosphate and 4 gms. potassium sulphate. Three young plants of rice per pot were transplanted on July 9, and the crop was harvested on October 10, 1928. The yieldes (dry matter) of grain and strow of control (mean of two pots) were 11.8 gms. and 24.0 gms. per pot respectively, and nitrogen contained in them were 0.1205 gm. and 0.1674 gm. respectively. From these increased yields and availabilities of added nitrogen, etc. are calculated as follows.

| Applied per pot | | Increased yields of total crop (dry weights in gms.) | Relative increases of total yields | Increased yields of grain (dry weights in gms.) | Relative increases of grain | % added N removed in crop | Relative availabilities of added N |
|---|---|---|---|---|---|---|---|
| Ammonium Sulphate | 1.0 gm. N | 82 4 | 100 | 24.6 | 100 | 80.2 | 100 |
| | 0.5 gm. N | 55.5 | 100 | 17.5 | 100 | 71.2 | 100 |
| | 0.25 gm. N | 27.9 | 100 | 11.9 | 100 | 77.6 | 100 |
| Dicyanodiamide | 1.0 gm. N | 66.4 | 80.5 | 31.9 | 137.8 | 65.8 | 82.0 |
| | 0.5 gm. N | 59.2 | 106.6 | 27.4 | 156.5 | 74.0 | 103.9 |
| | 0.25 gm. N | 33.9 | 121.5 | 17.4 | 146.2 | 95.6 | 123.1 |

The surpassing manurial value of dicyanodiamide over ammonium sulphate is generally observed in above results, and is more remarkable in the increased yields of grain. But, under the natural paddy-field condition the manurial value of dicyanodiamide would not be so high as in pot culture experiments. For dicyanodiamide is difficult to be retained in the soil, and well be easily washed out by drainage water through the soil.

The author considers that the exclusive fertilizing value of dicyanodiamide on paddy-rice, as against its harmful effect on most plants, is due briefly to the following facts:

(a) Paddy-rice has a somewhate greater resistance towards the harmful action of dicyanodiamide than dry-field (upland) crops.

(b) The indirect harmful action of dicyanodiamide, due to the retarding effect upon nitrification, does not affect the paddy-rice, as the nitrification does not occur essentially in the water-logged paddy-soil and is not needed on the paddy-rice culture.

(c) Under the soil condition of water-logged paddy-field in summer season, when the paddy-rice is grown, the dicyanodiamide decomposes so moderately that the formation of ammonia from it is well in accord with the growth of paddy-rice. Dicyanodiamide is made accessible by the paddy-rice only when it is transformed to ammonia. The direct assimilation of dicyanodiamide by the paddy-rice appears difficult, and necessitates further investigation.

Consequently, the secondary harmful after-effects of the application of commercial cyanamide, due to the dicyanodiamide which may be produced from cyanamide, are likely to be only slight or entirely absent in paddy-rice culture, although it is frequently experienced in dry-field crop culture.

# ON THE OCCURRENCE OF A NEW AMINO ACID IN WATERMELON, CITRULLUS VULGARIS, SCHRAD.

By

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There are a number of investigations regarding the constituents of watermelon, but nitrogenous compounds of it are scarcely described. So the author studied them particularly and isolated a new amino acid and named it "Citrullin".

The analyses of the free compound as well as that of its copper salt have shown that the empirical formula should be $C_6H_{13}N_3O_3$.

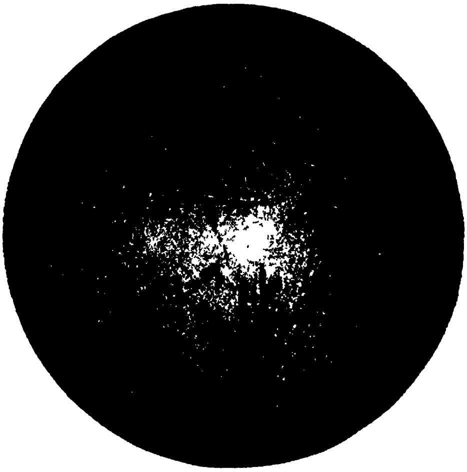
The isolation of this compound was carried out as follows: ——

The pressed juice of watermelon was filtered and the clear colourless liquid was evaporated in vacuum. The resulting syrup was dissolved in water to 1 L. and a concentrated basic lead acetate solution was added to it as a preliminary precipitant. The nitrogenous constituents obtained from the filtrate with Neuberg's reagent (mercuric acetate, sodium carbonate, and alcohol) was again treated with phosphotungstic acid. The filtrate from the phosphotungstic acid precipitate, that is, the monoamino acid fraction contained "Citrullin". The copper salt obtained from this fraction was violet thin prisms, a little soluble in hot water and decomposed at 257~8°. The yield of it was 3.4 g. from 2 kg. syrup. The free amino acid obtained from the copper salt is colourless thin prisms decomposing at 202°, but 205~6° in the sealed capillary. It is easily soluble in water but not at all in pure ethyl and methyl alcohols, ether, and the other organic solvents. The aqueous solution is nearly neutral to litmus, and possessed a slightly sweet taste. Showing no rotatory power, this compound seems to be racemic. It is precipitated in a concentrated solution with phosphotungstic acid, but the precipitate is easily soluble in the excess of the reagent. It is also precipitated with mercuric salts such as acetate, nitrate, sulphate, and chloride in an alkaline solution; but not with phosphomolybdic acid, tannin, and basic lead acetate.

The free compound gives reactions with ninhydrin, Ehrlich's and Schiff's reagents and with phenol and sodium-hypochlorite; but it does not give following reactions; Biuret, Diazo, Millon's, Jaffe's, Sakaguchi's, Folin's, Adamkiewicz's, and Mörner's reactions. This compound is not hydrolysed with sulphuric or hydrochloric acid, and has no properties of an anhydride or a peptide.

Two third of its nitrogen is liberated by the Van Slyke's method, and one third is titrated by the Sörensen's formol method. And so this has two amino and one carboxyl groups.

Citrullin (δ-Carbamyl ornithin). 1 : 165.



Copper salt of Citrullin. 1 : 770.

The author, taking into consideration the fact that there exist pairs of amino acids from the difference of one oxygen molecule, alanine and serine, phenylalanine and tyrosin, proline and oxyproline, glutamic acid and oxyglutamic acid and lysine and oxylysine, first suspected that the new amimo acid might be a derivative of arginin, of which one amino group was substituted by an oxy group. On preparing arginic acid, and comparing its properties with the above compound, a big difference was noticed, especially, in the fact that the former is decomposed by acids and the latter by weak alkalies. The new compound is not, therefore, surely arginic acid. From the following reasons, the author proposes the formula.

$$NH_2\text{-}CO\text{-}NHCH_2CH_2CH_2CH(NH_2)COOH$$

to the compound now in consideration.

(1) α-Amino δ-oxyvalerianic acid gives a copper salt, but δ-amino α-oxyvalerianic acid does not.

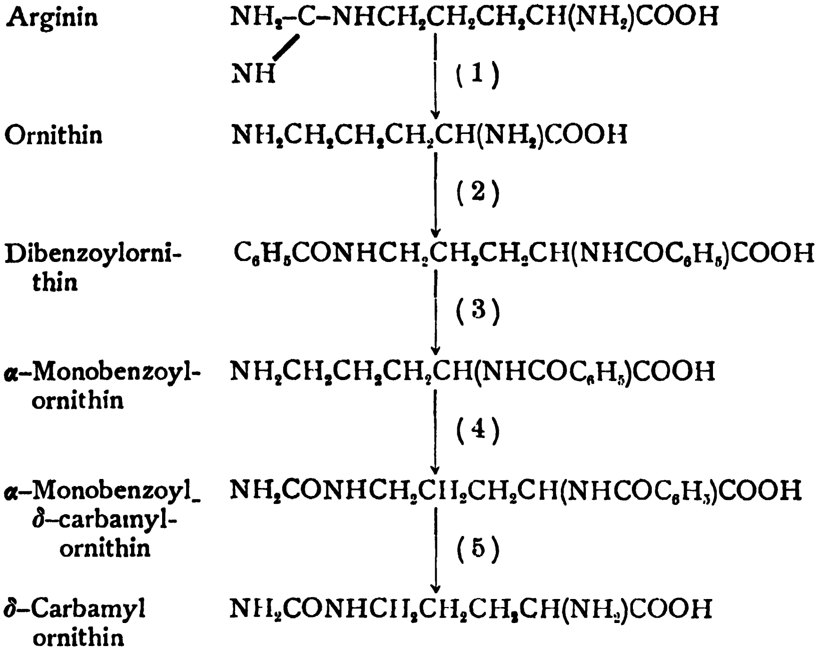
(2) Arginin gives a copper salt, but arginic acid does not. The new amino acid must have, in all probability, an α-amino group.

(3) The carbamyl group attached to α-amino group forms an anhydride by mineral acids, but the new amino acid does not.

(4) By the action of alkalies, the new amino acid gives off ammonia and is converted into ornithin.

(5) It gives reactions of urea such as Ehrlich's and Schiff's reactions.

Hereupon, the author synthesized the compound of the above constitution by the following five steps.



Arginin $NH_2-\underset{NH}{C}-NHCH_2CH_2CH_2CH(NH_2)COOH$

$\downarrow$ (1)

Ornithin $NH_2CH_2CH_2CH_2CH(NH_2)COOH$

$\downarrow$ (2)

Dibenzoylornithin $C_6H_5CONHCH_2CH_2CH_2CH(NHCOC_6H_5)COOH$

$\downarrow$ (3)

α-Monobenzoylornithin $NH_2CH_2CH_2CH_2CH(NHCOC_6H_5)COOH$

$\downarrow$ (4)

α-Monobenzoyl-δ-carbamylornithin $NH_2CONHCH_2CH_2CH_2CH(NHCOC_6H_5)COOH$

$\downarrow$ (5)

δ-Carbamyl ornithin $NH_2CONHCH_2CH_2CH_2CH(NH_2)COOH$

The synthesised product showed the same crystal form and the same decomposition point with the natural one, and properties and reactions of the both were same in every respect.

# Errata

Bulletin of the Agricultural Chemical Society of Japan. Vol. 6, Nos. 1–5.
On the Changes of Cyanamide in the Soil. Part I. By Hisaji Murata.

| Page. | Line. | Error | Correct. |
|---|---|---|---|
| **26** | **14** | **Mattew's** | **Matthews'** |
| ″ | **27** | **nutralizing** | **neutralizing** |
| **27** | **30** | **paper.)** | **paper).** |
| **28** | **17** | **condition** | **conditions** |
| ″ | **18** | **per 100 mgs.** | **per 100 gms.** |
| ″ | **28** | **experiment** | **experiment,** |
| **29** | **11** | **dose not** | **does not** |
| ″ | **19** | **matorially** | **materially** |
| ″ | **35** | **rapidly** | **rapidly,** |
| ″ | **37** | **intersting** | **interesting** |
| **30** | **2** | **moderately to** | **moderately** |
| ″ | **18** | **strow** | **straw** |
| ″ | **21** | **increased** | **the increased** |
| **31** | **5** | **well be** | **will be** |

Vol. 6, Nos. 6–9. Biochemical Studies on *Salmonidae*. V–VI.
By Hidesaburo Sekine, assisted by Yuji Kakizaki.

| | | | |
|---|---|---|---|
| **36~43** | | **Lipoid** | **Lipid** |

# BIOCHEMICAL STUDIES ON BONE, SKIN, HIDE AND SHEAL.

## 1. SEXUAL DIFFERENCES IN THE COMPOSITION OF BONE. (PRELIMINARY REPORT)

By

HIROMICHI HARA.

(Received 10th June 1930)

The composition of proteins, fats and others are different by sex. So the bone must also differ in its composition. Not only the contents of phosphorus and calcium, but the structure of bone protein, collagen, and the state of combination with calcium must differ.

On this opinion, the author studied the composition of bone from the standpoint of sex.

*Preparation of bones.*

The bones were removed from the body immediately after death. The adherent soft tissues were dissected from the bones which were then crushed into small fragments. The crushed bones were immediately extracted in a Soxhlets extraction apparatus with a mixture of ethyl-alcohol and ether for about 5 hours. Before beginning the extraction this mixture was made alkaline with sodium hydroxyde. And extracting temperature not exceeding 38-degrees. The bones were then pulverized finally and passed through an 80 mesh shieve. The preparation of the material for analysis was completed within 24 hours after removed of the specimen from the body.(1)

*Determination of total nitrogen and ash.*

Total nitrogen was determined by the Kjeldahl method, and the content of ash by the ordinary method.

The results showed that the contents of total nitrogen are higher in the male bone than the female.

*Determination of calcium and phosphorus.*

10mg. of powdered samples were weighed and transferred to a dry 10c.c. volumetric flask. 2c.c. of 1N HCl added and the mixture was then digested in a boiling waterbath for about 10 minutes. When it was cooled, 3c.c. of 20% trichloroacetic acid were added and the volume was made up to 10c.c. with dist. water. Aftor 30 minutes it was filtered.

2c.c. of the filtrate were used for the determination of calcium and the other 2c.c. of the filtrate for phosphorus. Calcium was determined by Kramer and Tisdall's method[2] and phosphorus by Bell-Doisy-Briggs' method.[3]

The datas showed that the contents of calcium were superior in femal, and the phosphorus were superior in male.

When these datas were calculated to N=100, the contents of both calcium and phosphorus were superior in femal to male. This shows that the ability of accumulation of nitrogen is inferior in female to male, while the contents of mineral matters in nitrogenous substances superior to male.

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(2) Kramer and J. Tisdall: Biol. Chem., 47. (1921).
(3) A. P. Briggs: J. Biol. Chem., 53. (1922).

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# BIOCHEMICAL STUDIES ON *SALMONIDAE*. V.

## ON THE INFLUENCE OF THE SUN-LIGHT ON CHEMICAL COMPOSITION OF FISH.

## I. ESPECIALLY ON ASSIMILATION OF CALCIUM AND NITROGEN AND ACCUMULATION OF LIPOIDS, BY "KIZAKI-KO-MASU", *ONCORHYNCHUS MASOU*, BREVOORT, IN LARVAL AND POST-LARVAL STAGES.

By

HIDESABURO SEKINE, assisted by YUJI KAKIZAKI.

(Received June. 6th., 1930)

"Kizaki-ko-Masu" or the common trout-landlocked were employed as experimental animals on this study was carried out at the Fishculture Station by the Lake of Kizaki, the Prefecture Nagano. 1000 eggs 37 days old after fertirization were divided into two parts in the same number, one of them fed in the hatchery (in the shade) and the other kept out of it (in the light), and the both fed on the same diet to see what effects are there.

78 and 88 larvae just absorbed their yolk-sacs in the both groups were respectively taken for the first analytical samples on the 18th. in February -the 25th. day from the beginning.

TABLE I. The influence of the light on the larvae just absorbed their yolk-sacs.

| | Live weight | Water | Solids | Ash | Calcium | Organic matter | Nitrogen |
|---|---|---|---|---|---|---|---|
| Shade | 296.7 | 247.9 | 48.82 | 3.642 | 0.923 | 45.18 | 5.37 |
| | 100.0 | 83.55 | 16.45 | 1.216 | 0.310 | 15.23 | 1.81 |
| | | | 100.00 | 7.46 | 1.89 | 92.54 | 11.00 |
| Light | 291.7 | 241.5 | 50.245 | 3.792 | 0.959 | 46.45 | 5.64 |
| | 100.0 | 82.78 | 17.22 | 1.300 | 0.329 | 15.90 | 1.93 |
| | | | 100.00 | 7.55 | 1.91 | 92.45 | 11.23 |

Table I indicates the analytical results of the samples mentioned above. These figures indicate that the sun-light brings already a sufficient effect chemically on young fry through the larval stage. By comparision with those fishes are shown in the table, the ones in the shade are havier in live weight but less in solids (both organic and inorganic, especially N & Ca) than those in the light. And these results coinside to the preceding report on fasting of salmons, *Oncorhynchus keta* Walbaum, in the light and dark.

The second examination was done on the 89th. day (May 18th.) since the first examination had been done. According to the results (see Table II) it may be seen that the light brings the better effects on both growth and number of the fishes survived.

TABLE II. The influence of the sun-light on the feedings and samples for chemical analyses.

| In the shade | Live weight (g.) | Number | Average weight | Samples for analyses.* | |
|---|---|---|---|---|---|
| | | | | Number | Average weight |
| Total weight | 167 | 121 | 1.38 | | |
| The items | | | | | |
| Large | 57 | 30 | 1.9 | 10 | 2019.4 |
| Medium | 76 | 55 | 1.38 | 22 | 1230.2 |
| Small | 34 | 36 | 0.943 | 19 | 788 0 |
| In the light | | | | | |
| Total weight | 254 | 247 | 1.03 | | |
| The items | | | | | |
| Large (specially) | 20 | 4 | 5.0 | 4 | 3950 |
| Large | 48 | 30 | 1.6 | 15 | 1401 |
| Medium | 177 | 195 | 0.908 | 20 | 849.7 |
| Small | 9 | 18 | 0.5 | 18 | 483.05 |

It is noticable phenomenon that there are remarkable differenciations in the rates of growth of the fishes feeding on the same condition, and it may be seen that the larger ones, as comparing with the smallers, rich generally in lipins but poor in nitrogen, as shown in Tables III and IV

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* Samples for analyses of the study are weighed after took off surface water of their bodies.

TABLE III. Results of analyses of the second examination.

| In the shade | Live weight (g) | Solids | Ash | Calcium | Nitrogen | Phos-phorus | Lipoids |
|---|---|---|---|---|---|---|---|
| Large | 2019.4 | 470.68 | 34.09 | 5.264 | 36.16 | 6.206 | 171.3 |
| | 100 | 23.349 | 1.725 | 0.261 | 2.255 | 0.357 | 8.48 |
| | | 100 | 7.40 | 1.12 | 9.657 | 1.524 | 36.33 |
| Medium | 1230.2 | 262.95 | 21.94 | 2.682 | 25.17 | 4.583 | 80.89 |
| | 100 | 21.38 | 1.783 | 0.218 | 2.046 | 0.373 | 6.58 |
| | | 100 | 8.34 | 1.02 | 9.57 | 1.743 | 30.76 |
| Small | 788.0 | 160.3 | 13.89 | 2.229 | 17.04 | 2.526 | 40.51 |
| | 100 | 20.35 | 1.762 | 0.283 | 2.163 | 0.332 | 5.15 |
| | | 100 | 8.66 | 1.39 | 10.63 | 1.631 | 25.30 |
| In the light | | | | | | | |
| Large (specially) | 3950 | 949.5 | 80.48 | 17.68 | 99.31 | 15.52 | 312.3 |
| | 100 | 24.04 | 2.037 | 0.448 | 2.514 | 0.392 | 7.91 |
| | | 100 | 8.475 | 1.86 | 10 46 | 1.635 | 32.89 |
| Large | 1401 | 314.1 | 26.56 | 4.70 | 29.77 | 5.075 | 107.3 |
| | 100 | 22.42 | 1.895 | 0.336 | 2.135 | 0.362 | 7.66 |
| | | 100 | 8.435 | 1.49 | 9.48 | 1.616 | 34.15 |
| Medium | 849.7 | 181.25 | 13.74 | 3.62 | 18.25 | 1.215 | 56.87 |
| | 100 | 21.33 | 1.62 | 0.427 | 2.147 | 0.143 | 6.69 |
| | | 100 | 7.58 | 2.00 | 10.07 | 0.670 | 31.40 |
| Small | 483.1 | 96.28 | 7.18 | 2.032 | 11.00 | 1.414 | 28.28 |
| | 100 | 19.95 | 1.49 | 0.421 | 2.278 | 0.210 | 5.86 |
| | | 100 | 7.45 | 2.11 | 11.42 | 1.052 | 29.36 |

The average values of these samples are calcurated and concised in Table IV.

On the average weights of the fishes in the shade and in the light the former is greater than the latter, and the former accumulates more lipoids but less in calcium assimilation.

TABLE IV. The average chemical compositions of all the samples in the both conditions.

| | Live weight (mg.) | Solids | Ash | Calcium | Nitrogen | Lipoids |
|---|---|---|---|---|---|---|
| Shade | 1258 | 251.2 | 22.15 | 3.118 | 26.80 | 93.58 |
| Light | 938.5 | 201.4 | 14.86 | 3.810 | 19.28 | 63.53 |
| Shade | 100 | 21.56 | 1.762 | 0.248 | 2.130 | 7.439 |
| Light | 100 | 21.46 | 1.647 | 0.406 | 2.161 | 6.767 |

The last examination was done on the 120th. day (September 14–15th.) with the similar results to those in the second time, as are shown in Table V.

TABLE V. The influence of the sun-light on the feedings and the samples for analyses.

| | Shade | | | Light | | |
|---|---|---|---|---|---|---|
| | Live weight (g.) | Number | Average weight (g.) | Live weight (g.) | Number | Average weight (g.) |
| Total weight | 183.98 | 30 | 6.133 | 277.86 | 77 | 3·73974 |
| The items | | | | | | |
| Large | 60.38 | 7 | 8.628 | 90.75 | 14 | 6.482 |
| Medium | 95.84 | 16 | 5.990 | 122.07 | 33 | 3.6991 |
| Small | 27.76 | 7 | 3.966 | 52.08 | 30 | 1.736 |

The medium fishes in the former and the large ones in the latter both of which are almost the same weight were analysed, and the results are given in Table VI. According to the figures the fishes in the light, as compared with those in the shade are rich in solids, ash, calcium, magnesium and nitrogen while are poor in lipoids and lipoids-nitrogen.

TABLE VI. Analytical results of the all fishes in the both condition on the end.

| | Live weight (mg.) | Solids | Ash | Calcium | Magnesium | Nitrogen | Lipoids | N in lipoids |
|---|---|---|---|---|---|---|---|---|
| Shade | 5990 | 1391 | 115.3 | 21.96 | 1.976 | 141.4 | 427.6 | 2.103 |
| | 100 | 23.22 | 1.921 | 0.3665 | 0.033 | 2.360 | 7.139 | 0.035 |
| | | 100 | 8.288 | 1.578 | 0.142 | 10.148 | 30.74 | 0.151 |
| Light | 6482 | 1437 | 139.9 | 23.96 | 4.342 | 153.8 | 339.8 | 0.956 |
| | 100 | 25.33 | 2.466 | 0.4224 | 0.013 | 2.712 | 5.991 | 0.0174 |
| | | 100 | 9.735 | 1.667 | 0.171 | 10.725 | 23.65 | 0.0686 |

## Summary and Conclusion.

1) The influence of the sun-light on the eggs, larvae and fry of Oncorhynchus masou (Brevoort) are studied.

2) The intensity of the sun-light may be effected already upon their chemical compositions in the fry through the oval and larval stages. The fish fed in the lighter place (in the open air out of the hatchery), as compared with the darker place (in faint light inside of the house), are rich in solids, inorganic matters (especially calcium) and nitrogen and are poor in fatty substances.

3) There occur the considarable differenciation in weights of the fish in a group fed on the same diet regardless of the light. And the larger ones are in generally rich in solids especially fatty substances.

4) From these facts, it is concluded as follows:– This species of fishes *Oncorhynchus masou* (Brevoort) may be already fond of the light place on the youngest days. The fish therefore fed in the open air assimilates the more

calcium and nitrogen while that in doors accumulates surplus bodily fats,— these results are the similar to the cases in the preceding report on fasting of salmons fry *Oncorhynchus keta* (Walbaum[1]) in light and darkness.— and the larger ones in a group accumulate in generally the more debot fats without regard of the qauntity of the light.

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(1) H. Sekine (1929): Biochemical Studies on Salmonidae. IV. Bul. of the Agr. Chem. Soc. of Japan. Nos. 61–63, 63.

# BIOCHEMICAL STUDIES ON *SALMONIDAE.* VI.

## ON THE SUN-LIGHT ON CHEMICAL COMPOSITION OF A FISH. II. ESPECIALLY ON ASSIMILATION OF CALCIUM AND NITROGEN AND ACCUMULATION OF LIPOIDS BY RAINBOW TROUT'S FRY, *SALMO IRIDIUS* GIBBON.

By

HIDESABURO SEKINE, assisted by YUJI KAKIZAKI.

(Received June. 6th., 1930)

The same experiments as had been reported in the preceding paper were repeated with the rainbow trout.

2000 young fry of rainbow trouts, their yolk-sacs just absorbed on the end of June, were divided into two groups which kept inside and outside of the hatchery at Kizaki, Nagano. The fry in the same stage were analyzed and their chemical compositions are illustrated in the following table.

TABLE I. Chemical compositions of rainbow trouts soon after absorbed their yolk-sacs.

| Live weight (mg.) | Solids | Ash | Calcium | Magnesium | Nitrogen | Lipoids | N. in-lipoids |
|---|---|---|---|---|---|---|---|
| 150 | 26.67 | 1.846 | 0.166 | 0.9365 | 3.255 | 3.865 | 0.0781 |
| 100 | 17.68 | 1.231 | 0.111 | 0.0244 | 2.170 | 2.576 | 0.0414 |
| | 190.00 | 6.964 | 0.626 | 0.1377 | 12.296 | 14.566 | 0.2340 |

On the 14–15th. in September (the 69–70th. day) all the fishes surviving the full experimental period were caught for examination the results of which are summarized in Table II.

TABLE II. The influence of the sun-light on the fishes in the different conditions, and samples for analyses.

| | In the shade | | | In the light | | |
|---|---|---|---|---|---|---|
| | Live weight (g) | Number | Average weight | Live weight (g.) | Number | Average weight |
| Initial | 152 | 1000 | 0.852 | 150 | 1000 | 0.150 |
| Final | 227 | 492 | 0.461 | 240 | 357 | 0.695 |
| Samples for analysis; | | | | | | |
| Large | 35.819 | 42 | 0.8726 | 42.340 | 50 | 0.8468 |
| Small | 87.620 | 196 | 0.4470 | 46.580 | 100 | 0.4658 |

The two kinds of analytical samples weighing almost the same in averages as shown in Table II. The results of the analyses are summarized in Table III.

TABLE III. The chemical compositions of the rainbow trouts fry which fed inside and outside of the hatchery for about 70 days.

| In the shade | Live weight (mg.) | Solids | Ash | Calcium | Magnesium | Nitrogen | Lipoids | N in lipoids |
|---|---|---|---|---|---|---|---|---|
| Large | 853.5 | 180.5 | 10.88 | 1.194 | 0.249 | 19.05 | 50.21 | 0.1408 |
| | 100.0 | 21.14 | 1.276 | 0.1399 | 0.0291 | 2.232 | 5.88 | 0.0165 |
| | | 100.00 | 6.030 | 0.6614 | 0.1377 | 10.554 | 27.82 | 0.0780 |
| Small | 545.3 | 107.05 | 7.134 | 0.733 | 0.137 | 10.72 | 26.40 | — |
| | 100.0 | 19.65 | 1.308 | 0.128 | 0.0251 | 1.966 | 4.84 | — |
| | | 100.00 | 6.657 | 0.6838 | 0.1277 | 10.006 | 24.64 | — |
| In the light | | | | | | | | |
| Large | 846.8 | 171.45 | 10.73 | 1.279 | 0.228 | 18.66 | 44.47 | 0.0849 |
| | 100.0 | 20.24 | 1.267 | 0.1509 | 0.0270 | 2.204 | 5.252 | 0.0100 |
| | | 100.00 | 6.257 | 0.7437 | 0.1331 | 10.884 | 25.884 | 0.0496 |
| Small | 465.8 | 85.42 | 6.267 | 0.635 | 0.131 | 9.01 | 17.73 | — |
| | 100.0 | 18.34 | 1.346 | 0.1364 | 0.0282 | 1.979 | 3.806 | — |
| | | 100.00 | 7.337 | 0.7437 | 0.1537 | 10.790 | 20.75 | — |

## Discussion of Result.

Comparing the fishes in the hatchery with those in the open air, the former is lighter in total and average weights but is greater in number of fishes survived. In the next, on their chemical compositions the former contains the more solids, lipoids and lipoids-N but the less ash, calcium, and nitrogen than the latter. It may, therefore, be seen that the majourties of the results are similally to the preceding report(1).

## Conclusion.

The twice experiments on the influence of the sunlight on feeding fishes and their chemical compositions may be concluded as follows :–

Keeping trouts (in the youngest days) in the different conditions (in the inside and out of the hatchery) should be brought the different metabolisms in their bodies.

Then the fish in the open air assimilates high content of calcium and nitrogen while that in the shade contain smore deposit fats. Therefore, within the limits of general condition of feeding trouts, the more light brings the more assimilation of calcium and nitrogen but the less deposition of fatty substances. And the former case shows generally high value of total weight of the trouts survived than that of the latter.

According to the experimental results, it may be provable that it is not good to keeping the larvae and the young fry in the hatchery, which is always gloomy, for a long time after they have been hatched out.

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# THE IODOMETRIC DETERMINATION OF FURFURAL.

By

SHUIKU SASAKI.

(Received 5th , July, 1930.)

Among many methods determining furfural Pervier and Gortner's bromine method has called to the author's attention. The method is as follows :—— To the acid solution of furfural, some potassium bromide is added and titrated by N/10 potassium bromate. The end point of the reaction between bromine and furfural is determined by the time factor of colour change or by the degree of deflection of the needle of a galvanometer. This method is not reliable for they did not taken into account the effect of temperature upon the reaction. According to our experiments, bromine combines with more furfural at high temperature than at low temperature. Consequently, we modified their bromine method to an iodine method, and got more satisfactory results from the point of view of accuracy.

*Method* :—— Take about 6 per cent hydrochloric acid solution containing 5 to 18 mg. of furfural in 100 c.c. in a bottle, such as Merk's sulphuric acid bottle, which has a capacity from 250 to 300 c.c. Add 5 c.c. of 2 per cent potassium bromide, and exactly 10 c.c. of N/10 potassium bromate solution (containing 2.7838 g. potassium bromate in 1 litre of water). Seal tightly with a glass stopper. Allow it to stand for just 2 hours in a thermostat. Then add 10 c.c. of potassium iodide and titrate with N/50 sodium thiosulphate solution, of which 50 c.c. is equivalent to 50 c.c. of N/50 potassium bromate solution or to 10 c.c. of N/10 potassium bromate solution formerly added. When the end of the reaction approaches the brown colour of the solution changes to a pale yellow colour. At this time 2 c.c. of 0.5 per cent starch solution is added, and the titration is continued drop by drop, shaking the solution vigorously until the point of complete colourlessness is reached.

The following table shows the number of c.c. of N/50 potassium bromate which combines with 1 mg. of furfural at each temperature.

| Temp. | Bromate, c.c. | Temp. | Bromate, c.c. |
|---|---|---|---|
| 15 | 2.017 | 27.5 | 2.226 |
| 17.5 | 2.078 | 30 | 2.261 |
| 20 | 2.122 | 32.5 | 2.313 |
| 22.5 | 2.156 | 35 | 2.357 |
| 25 | 2.191 | | |

*Example* :—— When the temperature of the thermostat shows 20°, and 30 c.c. of N/50 sodium thiosulphate solution is required, the quantity of furfural is calculated as follows :——

50 c.c. − 30 c.c. = 20 c.c.

2.122 c c. : 1 mg. = 20 c.c. : $X$ mg.

$X = 20/2.122 = 9.42$ mg. furfural.

## LITERATURE.

( 1 ) Pervier and Gortner: The estimation of pentoses and pentosans. II. The determination of furfural. J. Ind. Chem , **15**, 1255, 1923.

# THE RESPIRATORY PROCESS OF THE SILK WORM AS AFFECTED BY TEMPERATURE, MOISTURE AND AIR CURRENT.

By

OTOMATSU FUJII.

(Received 10th, July 1930)

## ( 1 )

The silk worm is extremly sensitive to slight changes in its environment, especially temperature, moisture, and air current. Yet, despite the many papers dealing with this worm upon these factors, quantitative studies of its relations to the respiratory process have been rare and limited in scope.

## ( 2 )

Several years ago Crozier(1) reported the use of the critical thermal increment ($\mu$) for the characterization of biological process in various plants and animals whose velocities are a function of temperature and pointed out that in the case of oxidative phenomena critical increments were characteristically found to be of two, possibly three, types: $\mu=11{,}500$ and 16,100 or 16,700, and the first was commonly encountered above 15°C, the second below that temperature, but these relations might be reversed.

The author studied these relations of the respiratory process to other factors under as constant conditions as possible.

As shown in the following results, the respiratory process as affected by changes in temperature between the upper and the lower limit can be described experimentally in the terms of the follwing experimental equation

$$y=kt^p$$

in which $y$ is the amount of carbon dioxide produced, $t$ is the temperature, $k$ and $p$ are the constant.

TABLE I.

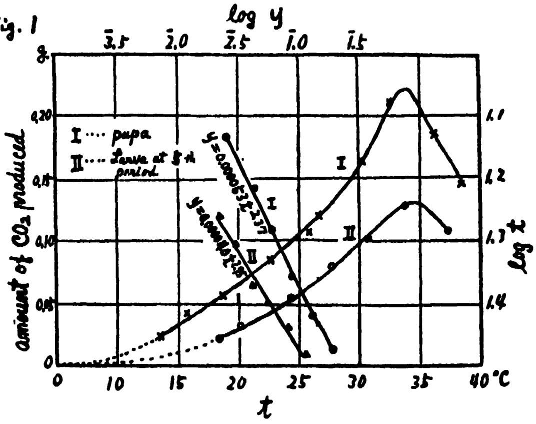
$CO_2$ production of pupa at different temperature.

| $t$ °C | $CO_2$ g. | $\mu=\frac{4.61\,T_1T_2(\log k_2-\log k_1)}{T_2-T_1}$ | $k=\frac{y}{t^{2.37}}$ | $p=\frac{\log\frac{y}{0.000053}}{\log t}$ |
|---|---|---|---|---|
| 13.4 | 0.0246 | 19981 | 0.000052 | 2.37 |
| 16.3 | 0.0416 | 20694 | 0.000056 | 2.39 |
| 19.2 | 0.0593 | 18068 | 0.000054 | 2.38 |
| 22.6 | 0.0842 | 15986 | 0.000052 | 2.36 |

| | | | | |
|---|---|---|---|---|
| 26.9 | 0.1239 | 16844 | 0.000051 | 2.36 |
| 30.2 | 0.1681 | 22784 | 0.000052 | 2.37 |
| 32.1 | 0.2124 | −7054 | 0.000057 | 2.39 |
| 36.2 | 0.1822 | −19772 | —— | —— |
| 38.4 | 0.1454 | —— | —— | —— |

From above results we see that the upper limit of the respiratory process is at about 33∼34°, and the lower limit at about 7∼8°, and the $\mu$ values suggested by Crozier are not constant. In his view, the same reaction exhibits different temperature coefficients when activated by different catalysts, and so the respiratory process must be activated by many different catalysts. But is this true? Further studies will solve these relations.

Fig. 1



## ( 3 )

The relations of moisture to the respiratory process can also be shown in terms of the following experimental equation

$$y = km^{-p}$$

in which $y$ is the amount of the carbon dioxode produced, $m$ is the relative humidity, $k$ and $p$ are the constant. The results were as follows.

TABLE. II.

CO₂ production of larva at different degrees of moisture.

| No. | Live weight | $t$ | $m$ | CO₂ produced | |
|---|---|---|---|---|---|
| | | | | measured | calculated ($y=0.213\,m^{-0.075}$) |
| | g. | °C | % | g. | g. |
| ♂35 | 101.6 | 24.4 | 71 | 0.1559 | 0.1551 |
| ♀35 | 101.6 | 24.5 | 78 | 0.1544 | 0.1540 |
| 〃 | 101.6 | 24.4 | 83 | 0.1531 | 0.1532 |
| 〃 | 101.6 | 24.4 | 98 | 0.1522 | 0.1513 |

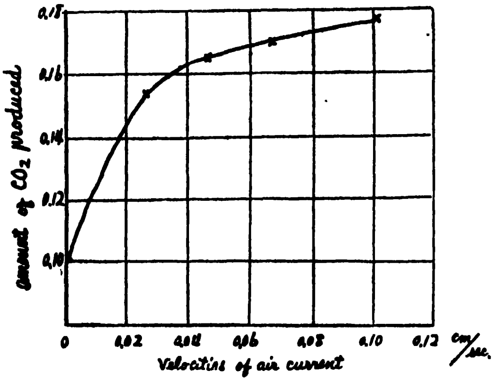
## ( 4 )

The continuous effects of temperature and moisture in the respiratory process were examined. As above mentioned, it is affected by temperature in the terms of $t^p$, in which $p$ is positive. But if observations are made during a continuous period it will be found that the higher the temperature the more rapid the decrease.

In the case of the effect of moisture, the same result was obtained.

### ( 5 )

The effect of the velocities of air current on the respiratory process was studied as far as possible at the same temperature and moisture. The following diagram shows the amount of carbon dioxide produced at the various velocities of air current.



Effect of velocities of air current on the respiratory process of larva at 5th period.

From this diagram we see that when there is no air current the amount of carbon dioxide produced is very much diminished, but as the air current increases the amount of carbon dioxide also increases, up to 0.1 cm/sec., the limit of our experimental apparatus.

## Summary.

(1) The effects of same environmental conditions on the respiratory process in the silk worm's pupa and larva at 5th period were studied.

(2) The effects of temperature, in the rang 14~32°C, and of moisture in the range 60~100%, can be described in terms of the following experimental equations.

$$y=kt^{p}$$
$$y=km^{-p}$$

in which $y$ is the amount of carbon dioxide produced, $t$ is the temperature, $m$ is the moisture, $k$ and $p$ are the constant.

(3) The upper critical temperature of the respiratory process is about 33~34°C, and its lower critical temperature is probably about 7~8°C.

(4) When there is the same temperature or moisture during a continuous period, the higher the temperature or the greater the moisture, the more rapid is the decrease in the production of carbon dioxide.

(5) Under the condition of no air current carbon dioxide production is very much diminished and it is increased up to 0.1 cm/sec. in proportion to the velocity of the air current.

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# ISOLATION OF XANTHOPHYLL FROM FRESH GREEN LEAVES.

By

K. SUZUKI and T. NISHIKAWA.

(Received 13th. July 1930).

Willstätter and Mieg (Ann. 355, I, 1907) were the first to isolate xanthophyll crystals from green leaves. They secured by their own method a yield of 12 grams of the crude crystals from 100 kgs. of air-dried nettles leaves. Jörgensen and Stiles (New Physiologist, Reprint, 1~180, 1917) isolated by another method 0.8 grams of the crude crystals of xanthophyll from 2 kgs. of air-dried nettle leaves.

According to these methods, fresh leaves must be first dried carefully either in the shade or by using some special rapid-drying apparatus which is fitted for drying of a considerable amount of fresh leaves in a short time and more over not getting the temperature above 40°C, since too high a temperature destroys a large percentage of the pigments present in the leaves.

Sometimes, these drying processes are very inconvenient, and hence we attempted to isolate xanthophyll crystals directly from fresh green leaves and obtained the satisfactory result by the following simple method.

5.1 kgs. of the fresh leaves of radish (Raphanus sativus, L.) are chopped about 1 cm. in length, divided into some portions and then put separately into an enamelled pot. Each portion is heated for a few minutes above 70°C with alcohol which is allowed to soak into the chopped fresh leaves. By this treatment, the fresh green leaves lose some parts of its moisture and will be changed to be macerated easily.

After heating, the alcohol is pressed off and the heated leaves are macerated in a motor. The alcohol which has been used for the heating of the first portion of the chopped fresh leaves can be used three successive treatments of heating of the fresh leaves, but not further.

Pigments are extracted from the macerated leaves with ether and the ether extract quickly sucked away. The extraction is repeated three times using fresh ether each time. By the extraction process, above mentioned, all of the leaf pigments will be extracted and the macerated leaves on the filter will be colorless. The ether extract is washed out with distilled water in order to remove traces of the alcohol, and dried over anhydrous sodium sulfate and then filtered.

The alcoholic solutions which were obtained by the heating of the chopped fresh leaves at the first treatment of this investigation are now combined, mixed with about equal volume of ether and, if neccessary, small amount of water is added at this time. The whole is shaken thoroughly and a great amount of water is added in order to cause a separation of the ether layer completely, and the aqueous alcohol layer which contains the brownish-yellow flavones is drawn off. The ether solution is washed out with water several times until no more green color is extracted, and dried over anhydrous sodium sulfate, then filtered.

All the ether solutions of the pigments are now combined, saponified with a small amount of methyl alcoholic potash by shaking thoroughly.

After complete saponification of chlorophylls, the ether solution is washed several times with water to remove the alkali-chlorophyllines completely. From this solution, ether is now evaporated in vacum at a room temperature. If reddish-green fluorescence is observed in the solution with the evaporation of the ether, a small amount of methyl alcoholic potash must be added again in order to saponify traces of chlorophylls, and the mixture is shaken well, and then the ether solution is washed several times with distilled water until the green color is completely removed. Then the ether is entirely evaporated off in vacuum at a room temperature, there remain xanthophyll crystals with a large quantity of reddish-brown resinous substances and some amount of carotin crystals.

About 500 c.c. of the light petroleum ether (b.p. 35~55°C) is poured to the residue and stirred well. On standing over a night at a room temperature, all the reddish-brown resinous substaces and the carotin crystals are dissolved but xanthophyll remains insoluble in the petroleum ether.

The xanthophyll crystals are separated by the filtration, washed with a little quantity of the petroleum ether, and then recrystalized twice from boiling absolute methyl alcohol.

We have obtained by this method 0.255 gram of pure xanthophyll crystals whose melting point is 174~175°C (uncorrected) from 5.1 kgs. of the fresh radish leaves.

The moisture content of the fresh radish leaves which were used for this investigation was 84.615% and that of air-dried state 2.955%.

Therefore, 5.1 kgs. of the fresh leaves can be converted into 935 grams of air-dried state. Consequently, the yield of 0.255 gram of the pure xanthophyll crystals from 5.1 kgs. of the fresh leaves must be said to be satisfactory result.

# STUDIES ON THE SAPONIN OF SOY-BEAN. PART II.

By

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(Received 13th., July 1930)

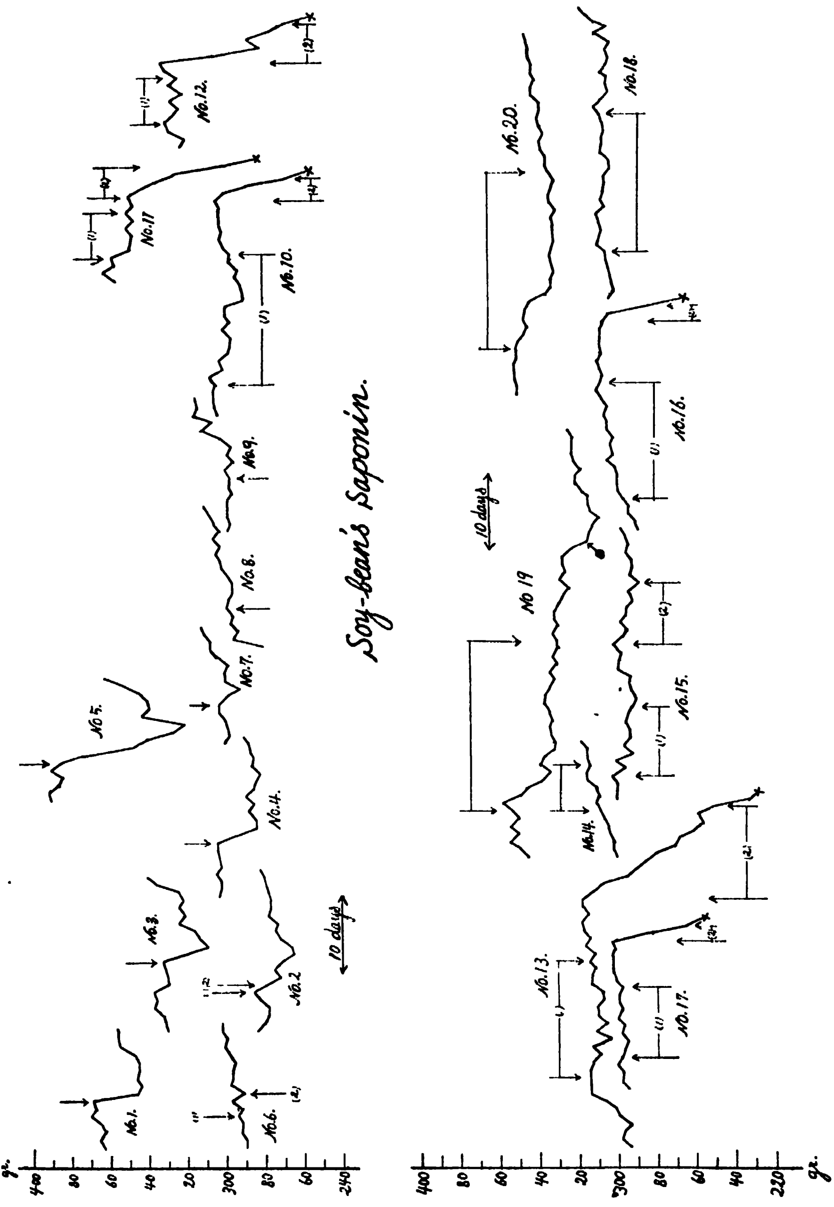
The author has received fortunately a large quantity of the alcoholic extraction of soy-bean from "Mantetsu Chūō Shikenjo", to which the author desires to express his thanks, and has been able to isolate the saponin easily as crystals. The Na-salt of saponin, decomposing at 259~260°, is prepared as hexagonal plate by neutralisation with Na-hydroxide from 70% alcoholic solution of free saponin.

The haemolytic and toxic powers of the saponin of soy-bean are studied comparing with Merck's saponin (pur. albiss) (aus der Wurzer der levantischen Saponaria).

The haemolytic power (against horse's blood) of the free saponin of soy-bean is observed in the concentration of 2000 : 1 and that of Na-salt is observed in the concentration of 50000 : 1, but the corpuscle is not destroyed completely in so high concentration such as 200 : 1. On the contrary Merck's saponin shows its haemolytic power in the concentration of 50000 : 1 and in the concentration of 25000 : 1 shows complete haemolysis.

The toxic power of soy-bean's saponin (free and Na-salt also), examined on pigions, is very weak though not being quite harmless, but that of Merck's saponin is very violent and a pigion dies by the hypodermic injection of 0.03 g. or by each deglutition of 0.05 g. during 2 days.

Fig 1.


Soy-bean's Saponin.
No.1.
No.2
No.3.
No.4.
No.5.
No.6.
No.7.
No.8.
No.9.
No.10.
No.11
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(1)
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10 days
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gr.
400
80
60
40
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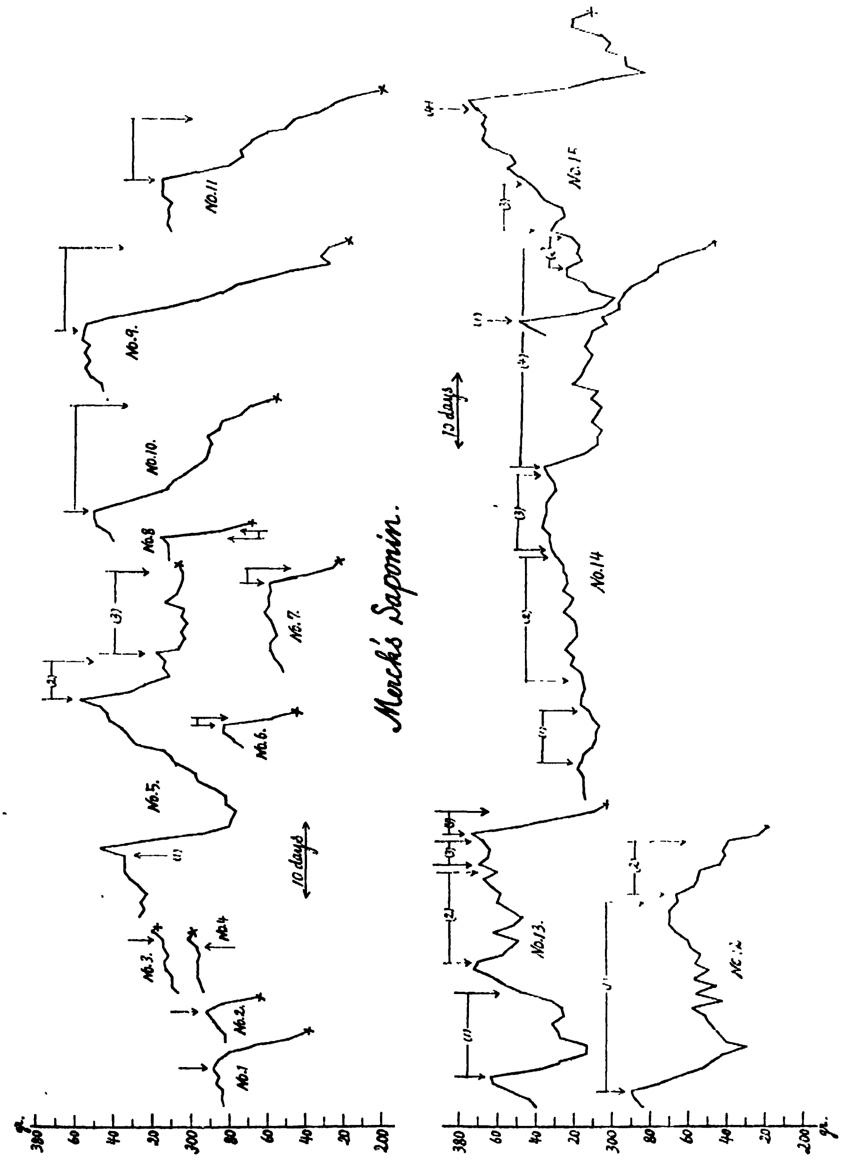
40
220

Fig 2.



Merck's Saponin.

# THE DISTRIBUTION OF PHOSPHORUS IN COW'S MILK AND THE SCHEME FOR THE SEPARATION OF PHOSPHATIDES

By

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(From the Institute of Agricultural Chemistry, College of Agriculture,
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(Received 9th September 1930)

## A. The Distribution of Phosphorus in Cow's milk

In order to extract the phosphatides, it is necessary first to determine the distribution of phosphorus in cow's milk. Liquid milk can not be extracted with ether or with other organic solvents owing to its large content of water.

The substance applied in this experiment is the milk powder, dried by the Buflovak drum drier below 70°C. Its content of moisture is 4.498%, determined by the method of drying in the air bath of 105°C.

The content of total phosphorus was determined(5) in the ash of sample which was burned with a little of fusing mixture.

| | In 100 g. of milk powder | In 100 g. of dry matter |
|---|---|---|
| Total phosphorus | 0.745 g. | 0.781 g. |

The amounts of phosphorus, which are soluble in the various organic solvents, were determined by extracting with three solvents in the following orders.

(A) Ether - Acetone - Alcohol
(B) Acetone - Ether - Alcohol
(C) Alcohol - Ether - Acetone

### Experiment (A)

1) At first a pair of each 10 g. of sample was extracted with ether by the Soxhlet's apparatus for a week. After the extraction had been completed, the extract was freed from ether and weighed.

| | In 100 g. of milk powder | In 100 g. of dry matter |
|---|---|---|
| Total ether soluble matters | 3.609 g. | 3.779 g. |

2) The above extract was dissolved in a very little of ether. On adding of acetone to the ethereal solution, the white amorphous precipitates were produced. The precipitates were filtered through the filter paper and washed with acetene.

Then the acetone soluble matter was freed from acetone and weighed.

It was burned to ashes with a little amount of fusing mixture and the content of phosphorus was determined.

| | In 100 g. of milk powder | In 100 g. of dry matter |
|---|---|---|
| Ether and acetone soluble matters | 3.078 g. | 3.223 g. |
| Ether and acetone soluble P | 0.10 mg. | 0.105 mg. |

The ether and acetone soluble fraction contains 0.003% of phosphorus. Then it might be assumed that this fraction was contaminated with about 0.082% of phosphatides, assuming that the phosphatides contain 3.94% of phosphorus as the mixture of equal parts of dipalmityl-, distearyl-and dioleyl-lecithin.

The ether soluble but acetone insoluble matter was dissolved in ether, and filtered through the filter paper. After it was freed from ether and weighed, it was burned to ashes with a little of fusing mixture, and the content of phosphorus was determined.

| | In 100 g. of milk powder | In 100 g. of dry matter |
|---|---|---|
| Ether soluble but acetone insoluble matters | 0.531 g | 0.556 g. |
| Ether soluble but acetone insoluble P | 0.07 mg. | 0.073 mg. |

This fraction contains 0.013% of phosphorus, as phosphatides 0.332% assuming that the content of phosphorus in phosphatides is about 3.94% as the mixture of lecithins.

3) The residue which was completely extracted with ether was freed from ether, and was again extracted with acetone at 30~40°C by the Soxlhet's apparatus for 4 days.

After the extracts were freed from acetone, it was weighed. Then it was disssolved in a little amount of ether. The insoluble matter was separated and washed with ether and acetone. Acetone was added to the ethereal solution and divided into two parts, that is, the one is soluble in both ether and acetone, and the other is soluble in ether but insoluble in acetone. The residue which is insoluble in both ether and acetone, was dissolved in water.

The above three fractions were weighed and the contents of phosphorus were determined by the same method as the above process.

| | In 100 g. of milk powder | In 100 g. of dry matter |
|---|---|---|
| Ether and acetone soluble matters | 11.443 g. | 11.982 g. |
| Ether and acetone soluble P | 0.53 mg. | 0.55 mg. |
| Ether soluble but acetone insoluble matters | 0.580 g. | 0.607 g. |

| | | |
|---|---|---|
| Ether soluble but acetone insoluble P | 0.33 mg. | 0.35 mg. |
| Water soluble matters | 1.629 g. | 1.706 g. |
| Water soluble P | 0.64 mg. | 0.67 mg. |
| Total soluble matters | 13.652 g. | 14.295 g. |

4) The residue which was extracted with ether and acetone, was further extracted with the boiling absolute alcohol.

The alcoholic solution was evaporated to a little volume and extracted with ether. Much insoluble matter was remained, which was further extract-ed with acetone and alcohol. Total extracts were mixed and evaporated to a little volume and expelled almost all of the solvents. And again it was dissolved in ether. There remained some insoluble matter.

The clear ethereal solution was divided into two parts, that is, the one is soluble in both ether and acetone, and the other is soluble in ether but insoluble in acetone, by the same process as the above experiment. Total residue which is insoluble in all of ether, acetone and alcohol, was soluble in water.

| | In 100 g. of milk powder | In 100 g. of dry matter |
|---|---|---|
| Ether and acetone soluble matters | 0.423 g. | 0.443 g. |
| Ether and acetone soluble P | 4.11 mg. | 4.30 mg. |
| Ether soluble but acetone insoluble matters | 0.121 g. | 0.127 g. |
| Ether soluble but acetone insoluble P | 0.33 mg. | 0.35 mg. |
| Water soluble matters | 17.275 g. | 18.089 g. |
| Water soluble P | 16.97 mg. | 17.76 mg. |
| Total soluble matters | 17.819 g. | 18.658 g. |
| Total soluble P | 21.41 mg. | 22.42 mg. |

## Experiment (B)

1) At first a pair of each 10 g. of sample was extracted with acetone by the Soxhlet's extracting apparatus for 4 days. After the extraction had been completed, the extracts were freed from acetone and weighed.

| | In 100 g. of milk powder | In 100 g. of dry matter |
|---|---|---|
| Total acetone soluble matters | 15.988 g. | 16.741 g. |

2) The above extract was dissolved in a very little of ether. On addition of acetone to the ethereal solution, the white amorphous precipitates were produced. The precipitates were filtered through the filter paper and washed with acetone. Then the acetone soluble matter was freed from acetone and weighed. It was burned to ashes with a little of fusing mixture and the content of phosphorus was determined.

| | In 100 g. of milk powder | In 100 g. of dry matter |
|---|---|---|
| True acetone soluble matters | 13.720 g. | 14.366 g. |
| True acetone soluble P | 1.05 mg. | 1.10 mg. |

The true acetone soluble fraction contains 0.008% of phosphorus. Then it might be assumed that this fraction was contaminated with about 0.194% of phosphatides.

The residues from the acetone soluble matter were dissolved in ether, and filtered through the filter paper. After it was freed from ether and weighed, it was burned to ashes with a little of fusing mixture, and the content of phosphorus was determined.

| | In 100 g. of milk powder | In 100 g. of dry matter |
|---|---|---|
| Ether soluble but acetone insoluble matters | 0.829 g. | 0.868 g. |
| Ether soluble but acetone insoluble P | 0.75 mg. | 0.79 mg. |

This fraction contains 0.090% of phosphorus, as phosphatides 2.296% assuming that the content of phosphorus in phosphatides is 3.94%.

The residue which was insoluble in acetone and ether, was dissolved in water. After it was evaporated, its weight and the content of phosphorus was determined.

| | In 100 g. of milk powder | In 100 g. of dry matter |
|---|---|---|
| Water soluble matters | 1.439 g. | 1.507 g. |
| Water soluble P | 0.38 mg. | 0.40 mg. |

Almost all of this fraction may be sugar and inorganic substances.

3) The milk powder which was completely extracted with acetone, was freed from solvent by evaporating at 80°C and powdered. Then this was again extracted with ether by the Soxlhet's extracting apparatus for 3 days. After the extract was freed from ether, it was weighed. The content of phosphorus in this fraction was determined as the above process.

| | In 100 g. of milk powder | In 100 g. of dry matter |
|---|---|---|
| Ether soluble matters | 0.243 g. | 0.254 g. |
| Ether soluble P | 0.05 mg. | 0.05 mg. |

This fraction contains only 0.021% of phosphorus and 0.523% as phosphatide.

4) The residue which was extracted with acetone and ether, was further extracted with the boiling absolute alcohol.

The alcoholic solution was evaporated to a little volume and extracted with ether. Much insoluble matter was remained, which was further extracted with acetone and then with alcohol. Total extracts were mixed and evaporated to a little volume and again it was dissolved in ether. There was remained some insoluble matter.

The clear ethereal solution was divided into two parts, that is, the one is soluble in both ether and acetone, and the other is soluble in ether but insoluble in acetone, by the same process as the above experiment. Total residue, that is insoluble in all of ether, acetone and alcohol, was soluble in water.

| | In 100 g. of milk powder | In 100 g. of dry matter |
|---|---|---|
| Ether and acetone soluble matters | 0.253 g. | 0.265 g. |
| Ether and acetone soluble P | 4.12 mg. | 4.31 mg. |
| Ether soluble but acetone insoluble matters | 0.105 g. | 0.110 g. |
| Ether soluble but acetone insoluble P | 0.10 mg. | 0.10 mg. |
| Water soluble matters | 19.077 g. | 19.975 g. |
| Water soluble P | 17·82 mg. | 18.66 mg. |
| Total soluble matters | 19.435 g. | 20.350 g. |
| Total soluble P | 22.04 mg. | 23.04 mg |

## Experiment (C)

1) A pair of each 10 g. of milk powder was extracted with the boiling absolute alcohol for five times. The extract was clear but faintly colored with yellowish green. On cooling, much white amorphous substance was precipitated. The precipitates were separated by centrifusing and washed with alcohol.

2) Precipitates: The precipitates were dissolved in ether. At that time, only a little of insoluble matter was remained, and it was soluble in water. On adding acetone in the clear ethereal solution, the white amorphous substance was precipitated. This was separated and washed with acetone.

Thus, the white precipirates were dissolved into three parts, that is, the one which is soluble in ether but not in acetone, the one which is soluble in both ether and acetone, and one which is insolubie in both ether and acetone but soluble in water.

| | In 100 g. of milk powder | In 100 g. of dry matter |
|---|---|---|
| Ether and acetone soluble matters | 8.469 g. | 8.868 g. |
| Ether and acetone soluble P | 0.17 mg. | 0.18 mg. |
| Ether soluble but acetone insoluble matters | 1.047 g. | 1.096 g. |
| Ether soluble but acetone insoluble P | 0.15 mg. | 0.16 mg. |

| | | |
|---|---|---|
| Water soluble matters | 1.449 g. | 1.517 g. |
| Water soluble P | 0.91 mg. | 0.95 mg. |
| Total soluble matters | 10.965 g. | 11.481 g. |
| Total soluble P | 1.23 mg. | 1.29 mg. |

3) Cold alcohol soluble matters: The alcoholic solution was evaporated to a little volume and extracted with ether. The ethereal solution was freed from ether and again dissolved in ether. A little of insoluble matter was remained.

The clear ethereal solution was divided into two parts, that is, the one is soluble in both ether and acetone, and the other is soluble in ether but insoluble in acetone, by the same process as the above experiment.

The white syrupy matter that is insoluble in all of ether, acetone and alcohol, was soluble in water.

| | In 100 g. of milk powder | In 100 g. of dry matter |
|---|---|---|
| Ether and acetone soluble matters | 5.999 g. | 6.282 g. |
| Ether and acetone soluble P | 5.03 mg. | 5.27 mg. |
| Ether soluble but acetone insoluble matters | 0.093 g. | 0.097 g. |
| Ether soluble but acetone insoluble P | 0.52 mg. | 0.54 mg. |
| Water soluble matters | 18.693 g. | 19.573 g. |
| Water soluble P | 17.72 mg. | 18.55 mg. |
| Total soluble matters | 24.785 g. | 25.952 g. |
| Total soluble P | 23.27 mg. | 24.37 mg. |

4) The residue from the extraction with the boiling alcohol, was further extracted with ether by the Soxhlet's extracting apparatus for a week, and then with acetone for 4 days. There was no extractive matter.

## Conclusion

The above three experimental results are summarized as follows:

| Solvents | In 100 g. of milk powder | | | | | |
|---|---|---|---|---|---|---|
| | Experiment(A) Ether - Acetone - Alcohol | | Experiment(B) Acetone - Ether - Alcohol | | Experiment(C) Alcohol - Ether - Acetone | |
| | Substances (g.) | P (mg.) | Substances (g) | P (mg.) | Substances (g.) | P (mg.) |
| Ether and acetone soluble | 15.648 | 4.96 | 14.631 | 5.41 | 15.149 | 5.44 |
| Ether soluble but acetone insoluble | 1.290 | 0.76 | 1.232 | 0.94 | 1.194 | 0.70 |
| Water soluble | 19.794 | 18.44 | 21.482 | 19.06 | 21.091 | 19.51 |
| Total | 36.732 | 24.16 | 37.345 | 25.41 | 37.434 | 25.65 |

If phosphorus, soluble in ether of acetone, is assumed as that of lecithin assuming as the mixture of equal parts of dipalmityl–, distearyl– and dioleyl-lecithin, containing each 3.94% of phosphorus, it is calculated as the following data.

| Solvents | In 100 g. of milk powder | | |
|---|---|---|---|
| | Experiment (A) Ether - Acetone - Alcohol | Experiment (B) Acetone - Ether - Alcohol | Experiment (C) Alcohol - Ether - Acetone |
| Ether and acetone soluble matters (True fats) | 15.522 g. | 14.494 g. | 15.011 g. |
| Ether and acetone soluble matters (Phosphatides) | 0.126 g. | 0.137 g. | 0.138 g. |
| Ether soluble but acetone insoluble matters | 1.271 g. | 1.208 g. | 1.176 g. |
| Ether soluble but acetone insoluble matters (Phosphatides) | 0.019 g. | 0.024 g. | 0.018 g. |
| Water soluble matters | 19.326 g. | 20.998 g. | 20.596 g. |
| Water soluble matters (Phosphatides) | 0.468 g. | 0.484 g. | 0.495 g. |

Based on the above three experimental results, it is known the following evidence. The greater part of phosphatides in cow's milk are more easily soluble in warm alcohol or warm acetone than ether. And phosphatides which can not be extracted by the direct extraction of the milk powder with ether, become soluble in ether when milk powder is extracted with the same solvent, after it is extracted with warm alcohol or warm acetone and then all the solvent is removed. It is known that when all the solvent is removed from the first acetone extract of the milk powder and the resulting residue is again taken up in ether, a good deal of substance is precipitated from the ethereal solution by acetone. And when the milk powder is treated as above described, the same total soluble matter can be obtained even if extracted with three solvents in different orders.

## B. Scheme for Separation for Phosphatides

The presence of lecithin and cephalin in cow's milk was noted by many investigaters, however, not identified by the complete analysis, and there is still without any definite evidence as to the presence of other phosphatide. From the nature of the problem, however, we are still without any reliable method on the isolation of the different phosphatides of cow's milk.

Assuming that all of fats and lipoids which were found in various animal tissues and organs are also present in cow's milk, the experimental procedure should be schemed to separate the individual phosphatide in a chemically pure form following to the many procedures(1) that were planned for the investigations of animal tissues and organs. The various steps in the process are as follows :

The milk powder is throughly extracted with ether by the use of the Soxhlet's extracting apparatus. Fats, cholesterol, lecithin, cephalin, myelin, cuorin, cerebrosides, paramyelin, vesalthin, amidomyelin and sphingomyelin are soluble in ether. After the residue of ether extraction is freed from ether, it is extracted with alcohol at 60°C. Then, a part of lecithin, cholesterol, fats, cephalin, cerebrosides, myelin and sphingomyelin that remain insoluble in the preceding extraction with ether, are extracted with hot alcohol while protein, sugar and mineral matter remain insoluble in the residue.

The ethereal solution is concentrated to a small bulk and treated with excess of acetone, which precipitates the greater part of lecithin, cephalin, myelin, cuorin and cerebrosides and a part of paramyelin, amidomyelin, sphingomyelin and cholesterol, leaving fats, cholesterol and vesalthin in solution. The precipitate is redissolved in a small amount of ether and excess of absolute alcohol added. The addition of alcohol causes cephalin cuorin, cerebrosides, myelin, paramyelin, amidomyelin and sphingomyelin (Precipitate C) to separate, while lecithin remains soluble in the mother liquor (D) which is filtered.

On cooling the above warm alcohol extract, the greater part of cerebrosides, myelin and sphingomyelin is separated. This precipitates (A) may contain some part of cephalin, cuorin, paramyelin and amidomyelin. The greater part of lecithin, cholesterol and fats remain in the mothor liquor (B), which is filtered and may contain also some of cephalin.

## Treatment of Precipitate (A)

After repeated dissolving and precipitating in alcohol, the precipitate (A) is separated into the ether soluble part and the ether insoluble part. The ethereal solution is treated with excess of acetone and the resulting precipitate (AA) is separated from mother solution (AB) by filtration. The resulting precipitate (AA) is again treated with ether, and an insoluble residue is separated by filtration. The ethereal solution is precipitated by alcohol; and the precipitate (AAA) is separated from the mother solution (AAB) by filtration.

The first ether insoluble residue of precipitate (A) is treated with hot alcohol. The alcohol soluble part is allowed to cool and the resulting precipitate (AC) is separated from the mother solution (AD) by filtration.

## Treatment of Mother liquor (B)

The mother liquor (B) is now concentrated under reduced pressure until all the alcohol is removed. The residue is again taken up in alcohol and the insoluble substance (BA) is separated from the soluble part (BB). The insoluble substance (BA) is now dissolved in a small volume of water containing a little sodium chloride, and extracted with ether by shaking in the separatory funnel.

The ethereal solution is now concentrated under reduced pressure until all the ether is removed, the residue is treated with warm alcohol. The alcoholic solution is cooled down and the precipitate (BAA) which separate out, is separated from the mother liquor (BAB).

## Treatment of Precipitate (C)

The combined precipitates of fraction (C) and fraction (AAA) are treated with ether and divided into two fractions of the insoluble part (CA) and the soluble part (CB). The former contains cerebrosides, myelin, sphingomyelin, paramyelin and amidomyelin, and the latter contains cephalin and cuorin. After the fraction (CB) is evaporated to remove ether, extracted with alcohol at 60°C whereby cuorin(2) is contained in the insoluble part (CBA) and cephalin(3) in the soluble part (CBB), and a precipitate separates on allowing the mixture to stand in the cold.

## Treatment of Mother liquor (D)

Mother liquor (D) and mother liquor (AAB) are mixed and concentrated under reduced pressure until all the solvent is removed. The residue (DA) is taken up in ether in which sphingomyelin(4) remains insoluble. The ethereal solution (DB) is filtered and excess of absolute alcohol(4) added, which precipitates (DBA) cephalin leaving leithin and other substances in solution (DBB).

## Treatment of Solution (BB)

To the mixture of the soluble part (BB), the mother liquor (AB), the mother liquor (BAB) and the mother liquor (DBB), the alcoholic solution of cadmium chloride is added in excess, and the precipitate (BBA)(4) is separated from the mother solution (BBB).

As there is doubt as to the contamination of fats and cholesterol with this precipitate of phosphatides, they are treated with hot acetone. Then the cadmium chloride of lecithin and cephalin remain insoluble, and the acetone solution is allowed to cool when a deposit (BBAc) occured; fat and cholesterol remain soluble in the mother solution (BBAd).

When the cadmium chloride salts of phosphatides is treated with hot ether(4), cephalin cadmium chloride salt is dissolved, in which lecithin cadmium chloride salt remains insoluble. The ethereal solution is filtered off and allowed to cool, and the cephalin cadmium chloride (BBAe) separated from the ethereal solution by alcohol (BBAf).

The fraction of lecithin cadmium chloride is now extracted with hot benzene(4), the lecithin cadmium chloride is dissolved but amidomyelin remains insoluble. On cooling this extract, a precipitate of paramyelin(4) forms. The filtrate obtained from the above preapitate is treated with excess of alcohol and the resulting precipitate (BBAa) is separated from the mother solution (BBAb).

The fraction of amidomyelin and paramyelin is again treated with hot alcohol and a precipitate (BBAg) is separated from the mother solution (B BAh).

### Treatment of Mother liquor (BBB)

The mother liquor (BBB) is combined with the mother liquor (BBAf), (BBAb) and (BBAh) and concentrated under reduced pressure until all the alcohol is removed. The residue is again taken up in alcohol and the insoluble substance (BBBa) separated from the alcoholic solution (BBBb).

## Summary.

The distribution of phosphorus in cow's milk (milk powder) has been determined and the existence of much phosphorus in the fraction of phosphatides has been proved.

The experimental procedure has been schemed to isolate the individual phosphatide, assuming that all of fats and lipoids which were found in various animal tissues and organs are also present in cow's milk.

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(3) Maclean, Z. f. physiol. Chem., **57**, 304,(1908).
(4) Abderhalden, 'Biochem. Handlex. III, 232; 237; 238; 242..
(5) Tisdall: Journ. of biol. Chem., **50**, 329, (1922).

# ON TEA CATECHIN ISOLATED FROM GREEN TEA.

By

MICHIYO TSUJIMURA.

(Received September 8th., 1930).

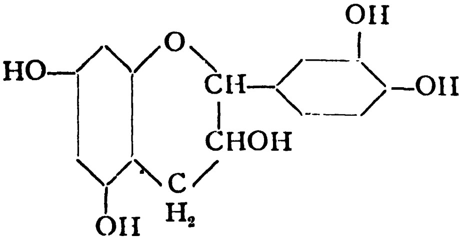
The tannin substance of tea (*Thea sinensis L.*) has frequently been investigated, but no decisive report has yet been pubilished. The auther has recently isolated a catechin from green tea in crystalline state and gave it the name "Tea Catechin". It forms colourless glistening prisms (Fig. 1) when crystallized from aqueous solution and melts at 237~238° (uncorr.) It gives also typical reactions for catechin. The analysis agrees with the formula $C_{15}H_{14}O_6$ which is now generally adopted for catechin. Its specific rotation is: $[\alpha]_D^{25} = -69°$. On acetylation it forms pentaacetyl derivative $C_{15}H_9O(OCOCH_3)_5$ m. p. 151~152°, thus showing the existence of five hydroxyl groups in the molecule. On the methylation, however, tetramethyl compound $C_{15}H_9O(OH)(OCH_3)_4$ m. p. 153~154° is obtained. This indicates that one hydroxyl group can be more difficultly methylated than others. Boiled with a concentrated alkali, phloroglucinol is formed as the chief decomposition product, and when the methylated product is cautiously oxidized by $KMnO_4$, veratric acid is formed. From these observations, tea catechin seems to have close resembrance to *l*-epicatechin isolated from acacia catechu by K. Freudenberg, the only difference being that the former contains no water of crystallization, when dried in the air, while the latter contains four molecules of water under the same condition.(1)

The comparison of tea catechin with other catechins obtained by different authors is as follows :

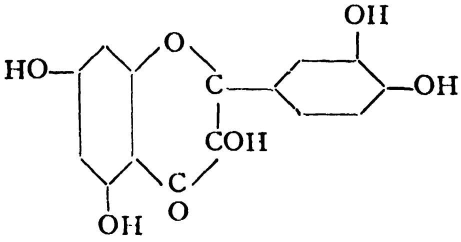
| | | | m. p. | Cryst. water | Secific rotation |
|---|---|---|---|---|---|
| A. G. Perkin: | Catechin C | $C_{15}H_{14}O_6$ | 235~237° | no | no description(2) |
| M. Nierenstein: | Isoacacatechin | $C_{15}H_{14}O_6$ | 237~238° | no | inactive(3) |
| K. Freudenberg: | *l*-Epicatechin | $C_{15}H_{14}O_6$ | 245° (corr.) | 4 mol | $[\alpha]_{Hg}$ yellow in alc.~69°(1) |
| The present author: | *Tea* catechin | $C_{15}H_{14}O_6$ | 237~238° | no | $[\alpha]_D$ in alc.~69° |
| K. Freudenberg: | Pentaacetyl *l*-epicatechin | $C_{15}H_9O(OCOCH_3)_5$ | m.p. 151~152°(4) | | |
| The present author: | Pentaacetyl *tea* catechin | $C_{15}O_9O(OCOCH_3)_5$ | m p. 151~152° | | |
| K. Freudenberg: | Tetramethyl *l*-epicatechin | $C_{15}H_9O(OH)(OCH_3)_4$ | m.p. 153~154°(4) | | |
| The present author: | Tetramethyl *tea* catechin | $C_{15}H_9O(OH)(OCH_3)_4$ | m.p. 153~154° | | |

As regards the chimecal constitution of catechin, the formula proposed by A.

G. Perkin and E. Yoshitake in 1902(2) seems to be the most reliable one and agrees best with various observations.



Catechin
(Perkin and Yoshitake)

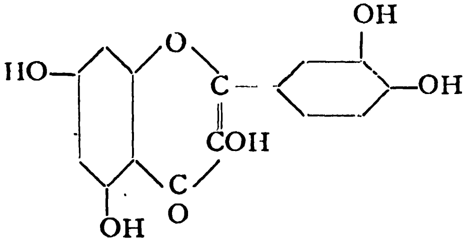


Quercetin

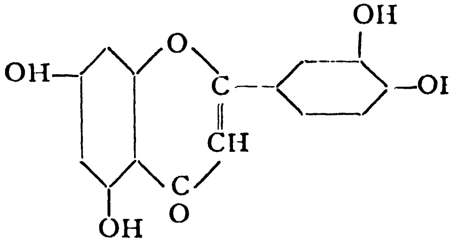
This assumption is chiefly based upon the close relationship existing between quercetin and catechin.

Leter, Freudenberg and his co-workers succeeded in transforming crynidin(5) and qurecetin(6) into catechin and gave further evidence in support of the Perkin's formula. They also studied the stereochemical relation of various kinds of catechins and classified(4) them into *d*-catechin, *l*-catechin, *d*, *l*-catechin, *d*-epicatechin, *l*-epicatechin and *d*, *l*-epicatechin. As there are two saymmetrical carbons in the Perkin's formula, the existence of such isomerides can easily be explained.

The author observed also that the absorption spectrum of tea catechin resembles to that of quercetin but not of luteolin. (See Y. Shibata and K. Kimotsuki's report.(7))



Quercetin
(Flavonol)



Luteolin
(Flavon)

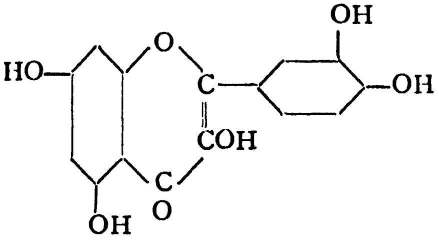
As this difference is simply due to the presence of one OH group attaching to the pyrone nucleus in quercetin, so it can be assumed that, the arrangement of hydroxyl groups in tea catechin is the same as that in quercetin.

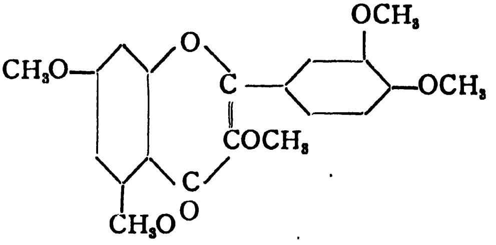
See Figs. of absorption spectra as follows:—

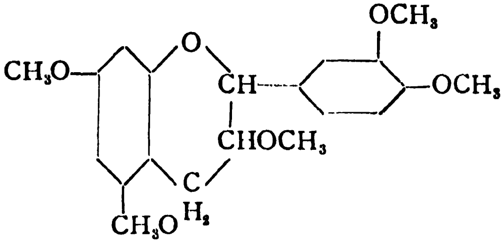
Fig. 3. Flavon (pentaacetyl quercetin)(7)
Fig. 4. Flavonol (querecetin)
Fig. 5. Tea catechin

Further evidence in support of this view is given by the auther, viz.;

when pentamethyl-eqercetin is hydrogenated in glacial acetic acid, using platinum oxide as catalyser, according to the following scheme:

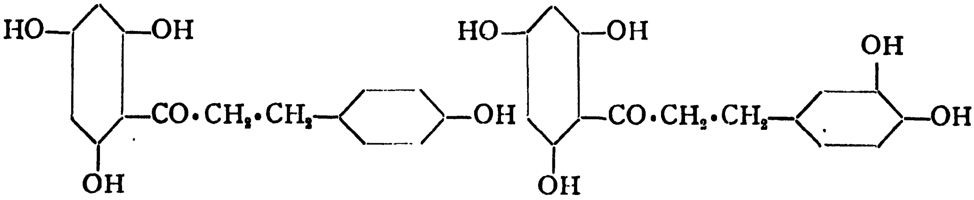

(1) Quercetin


(2) Pentamethyl-quercetin

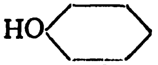
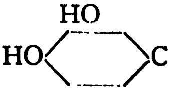

(3) Hydrogenated pentamethyl-quercetin

The hydrogenated product (3) shows exactly the same absorption spectrum (Fig. 6) with that of tea catechin and also with its methyl derivative. In this case, the methylation process seems to have no influence upon the absorption spectrum.

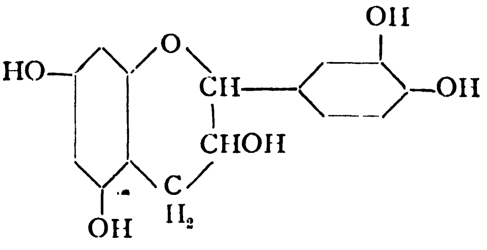
The question arises whether tea catechin might not be a series of phloretin, having the following formula:—


Phloretin ($C_{15}H_{14}O_5$)

Hypothetical formula for tea catechin ($C_{15}H_{14}O_6$)

However, such a hypothesis does not hold good because, (1) the absorption spectrum of phloretin is different from that of tea catechin, and further, (2) phloretin is decomposed into phloroglucinol and phloretic acid  $CH_2 \cdot CH_2COOH$, when boiled with concentrated potash but in the case of tea catechin such a substance as  $CH_2 \cdot CH_2 \cdot COOH$ could not be detected.

The author believes thus, that the catechin formula proposed by Perkin and Freudenberg has found further support by the spectrographic studies of the auther, and tea catechin, as one of the stereoisomers, must be represented by the same formula.



Tea catechin

## EXPERIMENTAL.

### (1) The Isolation of Tea Catechin from Green Tea.

The material used in this experiment was the green tea of superior quality, prepared in Kyoto prefecture. It was finely powdered and repeatedly extracted with boiling ethyl acetate. The extract was evaporated and the residue was taken up in water. The aqueous solution thus obtained was now treated with neutral lead acetate, warmed for a short time on a water-bath and filtered quickly. The filtrate was then decomposed with hydrogen sulphide, again filtered and evaporated under a diminished pressure. The brown syrup obtained in this way was extracted with alcohol, the alcoholic solution was evaporated and the remaining syrup was dissolved in hot water, filtered, and the filtrate was concentrated under a diminished pressure to a small volume. After standing it for a short time, a white crystalline mass separated out, which was collected, washed with water and after drying on a porous tile it was Soxhlet apparatus with chloroform. The insoluble brown coloured crystalline mass, was then dissolved in hot water and filtered. From the filtrate, colourless glistening prismatic crystals of tea catechin separated out, which after recrystallization from hot water melted constantly at 237—238° (uncorr.) The yield was about 0.14% of the green tea used.

### (2) Chief Properties of Tea Catechin.

The crystalline form of tea catechin obtained from aqueous solution is shown in Fig. 1, and that separated from alcoholic solution, in Fig. 2. The aqueous solution of tea catechin has an astringent taste. It gives a greenish blue colour with ferric chloride, which gradually changes to brownish yellow and finally forms a brown precipitate. When a few crystals of this substance are brought in contact with a pine shaving, moistened with concentrated hydrochloric acid, a reddish purple stain, characteristic of phloroglucinol is produced. It gives a reddish yellow colour with concentrated sulphuric acid, forming a precipitate. It is precipitated by lead acetate but not by gelatine. When bromine water is added to the aqueous solution a yellow precipitate is produced. When the aqueous solution is treated with lime water red colouration is first produced, forming a precipitate afterwards.

### (3) Estimation of Rotatory Power.

The estimation was carried out with 95% alcoholic solution of tea catechin.

$$[\alpha]_D = \pm \frac{\alpha \times 100}{c \times l}$$

| | | |
|---|---|---|
| $\alpha$ | observed rotation, | $-0.69°$ |
| $c$ | concentration, | 1% |
| $l$ | length of tube in decimeters, | 1 |

$$[\alpha]_D^{25} = -\frac{.69 \times 100}{1 \times 1} = -69°$$

### (4) Elementary Analysis.

The crystals obtained from aqueous solution were dried in the air to constant weight and analysed as follows:

| | | $CO_2$ | $H_2O$ | C. | H. |
|---|---|---|---|---|---|
| Sample I. | 3.412 mg. | 7.745 mg. | 1.568 mg. | 61.91% | 5.11% |
| 〃 II. | 2.565 〃 | 5.841 〃 | 1.218 〃 | 62.12 〃 | 5.28 〃 |
| Average | | | | 62.02 〃 | 5.20 〃 |
| Calc. for $C_{15}H_{14}O_6$ | | | | 62.06 〃 | 4.83 〃 |

The analytical result agreed with $C_{15}H_{14}O_6$ and showed that there was no water of crystallization.

### (5) The Determination of the Molecular Weight.

The determination of the molecular weight was carried out by Rast's camphor method⁽⁸⁾ with the following result:

$$m = \frac{c \times w \times 100}{dW}$$

| | | |
|---|---|---|
| $m$ | mol. wt. | |
| $c$ | const. | 400 |
| $w$ | subst. mg. | 11.3 |
| $d$ | depres. of m.p. | 9° |
| $W$ | solvent mg. | 159.7 |

$$m = \frac{400 \times 11.3 \times 100}{9 \times 159.7} = 314$$

Calc. for $C_{15}H_{14}O_6$ 290

## (6) Derivatives of Tea Catechin.

### (a) Acetyl Derivative.

Tea catechin (0.5 g.) was dissolved in acetic acid anhydride, to which sodium acetate anhydride (1.5 g.) was added and the mixture was heated on a water-bath for five hours. The acetylation product was then poured into water, filtered by means of a suction and washed with. After drying on a porous tile, it was dissolved in hot alcohol and filtered. After standing, white needle crystals were obtained: m.p. 151~152°. The analysis gave the following result.

| | | $CO_2$ | $H_2O$ | C. | H. |
|---|---|---|---|---|---|
| Sample I. | 4.574 mg. | 10.053 mg. | 2.152 mg. | 59.94% | 5.22% |
| 〃 II. | 3.550〃 | 7.849〃 | 1.480〃 | 60.23% | 4.63% |
| Average | | | | 60.14〃 | 4.93〃 |
| Calc. for | $C_{25}H_{24}O_{11}=C_{15}H_9O(OCOCH_3)_5$ | | | 60.0 〃 | 4.8 〃 |

### (b) Methyl Derivative.

The methylation was carried out by K, Freudenberg's method,(9) viz.; the crystal (1 g.) was dissolved in methyl alcohol (6 c.c.) and methylated in a usual way with dimethyl sulphate (3 c.c.) and 50% KOH (3 c.c.). The reaction product was poured into water (100 c.c.), filtered by means of suction and recrystallized from methyl alcohol. It froms convex-lens-like crystals: m.p. 153~154°. The result of analysis was as below:

| | | $CO_2$ | $H_2O$ | C. | H. |
|---|---|---|---|---|---|
| Sample I. | 2.954 mg. | 7.125 mg. | 1.706 mg. | 65.78% | 6.41% |
| 〃 II. | 2.844〃 | 6.826〃 | 1.938〃 | 65.46〃 | 6.40〃 |
| Average | | | | 65.62〃 | 6.41〃 |
| Calc. for | $C_{19}H_{22}O_6=C_{15}H_9O(OH)(OCH_3)_4$ | | | 65.89〃 | 6.35〃 |

The same methyl derivative was also obtained by methylating the acetylated product in the same manner as above.

$$C_{15}H_9O(OCOCH_3)_5 \longrightarrow C_{15}H_9O(OH)(OCH_3)_4$$

## (7) Decomposition of Tea Catechin with Concentrated Potash.

Tea catechin was boiled with 50% KOH at 180° for 30 munutes. After cooling, it was diluted with water, neutralized with an acid and then made weakly alkaline by adding sufficient sodium bicarbonate when it was filtered and extracted with ether. The ether extract was evaporated and treated with water. From this aqueous solution a quantity of crystalline plates was obtained, which melted at 214°.

It gave the reactions of phloroglucinol; mixed with pure phloroglucinol no depression of melting point was observed, so it was proved to be phloroglucinol.

The residue of the ether extract was acidified with acetic acid, and extracted with ether. From this extract a small quantity of needle-shaped crystals was obtained which gave a greenich blue colour with ferric chloride. But, owing to shortage of the material it was not further investigated.

### (8) Oxidation of Tetramethyl Tea Catechin with Potassium Permanganate.

The oxidation was carried out according to the method of Perkin(10). For this purpose, tea catechin tetramethyl ether was mixed with water, heated on a water-bath and a strong solution of permanganate was added drop by drop. The operation lasted two hours when the excess of permanganate was decomposed with sodium sulphite and filtered. The filtrate was neutralized with sulphuric acid and extracted with ether. From this etherial solution veratric acid separated out as fine prismatic crystals: m.p. 176~177°.

When pure veratric acid was mixed with the crystals obtained above, no depression of melting point was observed, hence it was proved to be veratric acid.

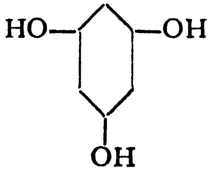
### (9) Preparation of Veratric acid.

For this purpose, the alcoholic solution of vanillin (2 g.) was mixed with alcoholic potash (0.74 g.) and evaporated to dryness. The dried residue was now treated with methyl alcohol (4 c.c.) and methyl iodide (2.5 g.) and heated on a water-bath. After cooling, the reaction mixture was extracted with ether, and the etherial solution was evaporated off. The residue thus obtained was mixed with some water and a 5% permanganate solution was added drop by drop, heated on a water-bath and filtered. The filtrate was now concentrated to a small volume and acidified with dilute hydrochloric acid, when veratric acid separated out as prismatic crystals, which after recrystallization from dilute alcohol melted constantly at 178°.

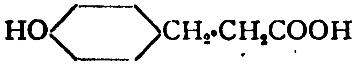
### (10) The Decomposition of Phloretin.

In order to compare tea catechin $C_{15}H_{14}O_6$ and phloretin $C_{15}H_{14}O_5$, phloretin was boild with 50% KOH in the same way as tea catechin was treated.

Phlorhglucinol



and phloretic acid



(m.p. 128°) were obtained.

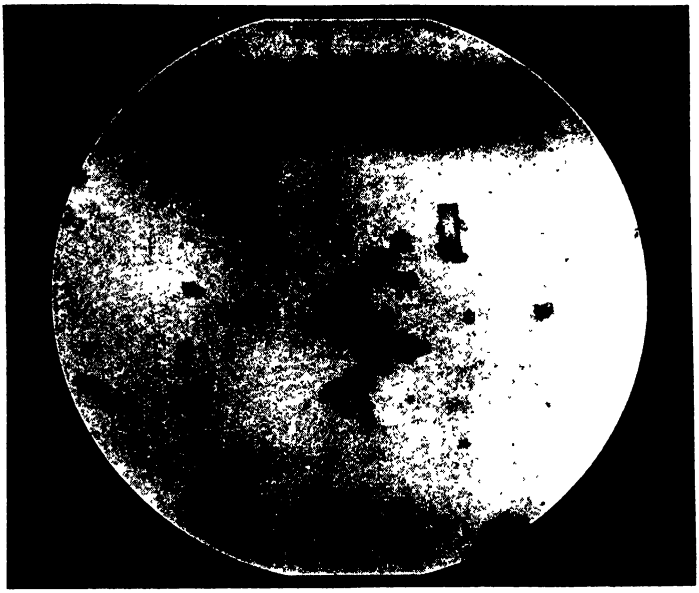


Fig. 1. Tea Catechin, from aq. sol.

1 : 100

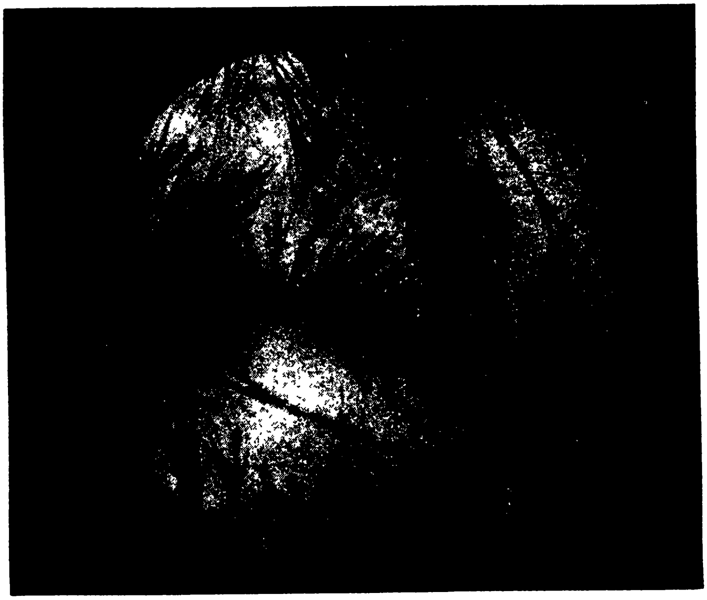


Fig. 2. Tea Catechin, from alc. sol.

1 : 100

$^1/_{10000}$ mol.

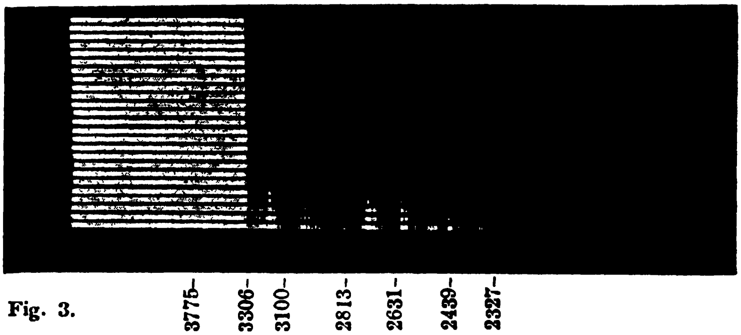


Fig. 3.

$^1/_{10000}$ mol.

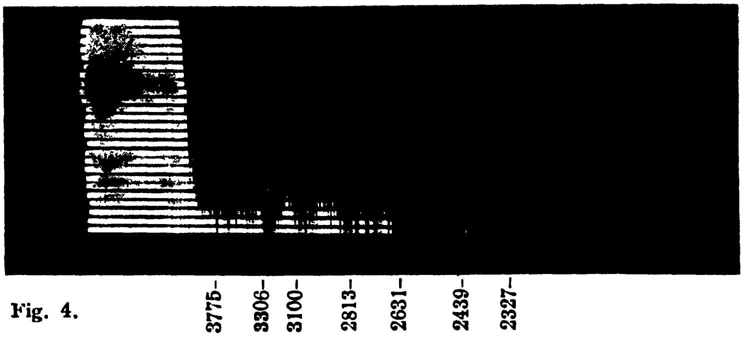


Fig. 4.

$^1/_{1000}$ mol.

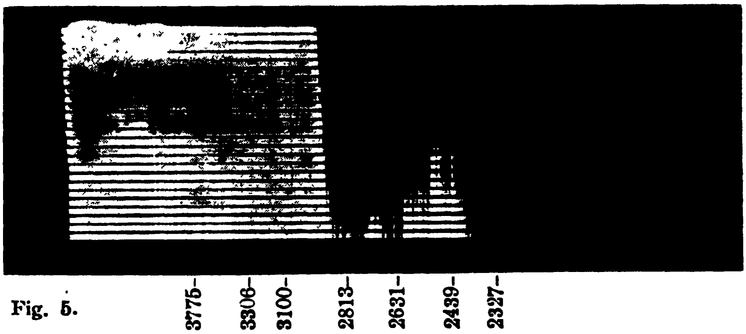


Fig. 5.

$^1/_{1000}$ mol.

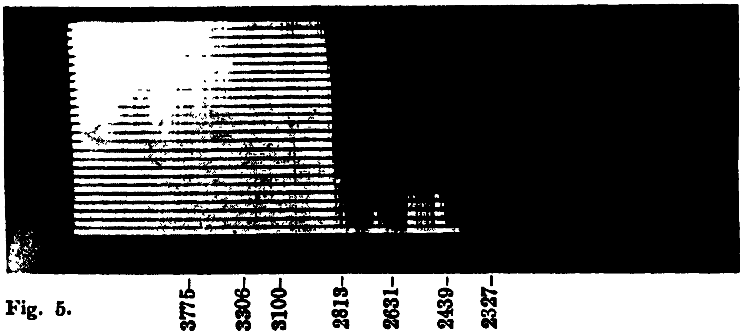
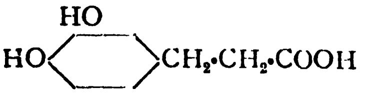


Fig. 5.

In the case of tea catechin, however, such a substance as  could not be obtained.

### (11) Methylation of Quercetin.

Quercetin was methylated in the same way as tea catechin and pentamethyl-quercetin was obtained: m.p. 147~148°.(11)
Analytical result was as follows:

| | | $CO_2$ | $H_2O$ | C. | O. |
|---|---|---|---|---|---|
| Sample I. | 3.236 mg. | 7.678 mg. | 1.575 mg. | 64.71% | 5.41% |
| 〃 II. | 2.416〃 | 5.699〃 | 1.166〃 | 64.33〃 | 5.36〃 |
| Average | | | | 64.52〃 | 5.39〃 |
| Calc. for | $C_{20}H_{20}O_7 = C_{15}H_5O_2(OCH_3)_5$ | | | 64.49〃 | 5.42〃 |

### (12) Hydrogenation of Pentamethyl-Quercetin.

Pentamethyl-quercetin (0.5 g) was dissolved in glacial acetic acid (5 c.c.) and hydrogenated in a usual way using platinum oxide(12) (0.1 g.) as catalyser, the operation lasted for 8 hours.

The absorpiion spectrum of the hydrogenated product is shown in Fig. 6.

The author expresses sincere thanks to Prof. U. Suzuki for his kind direction throughout this work and to Mr. S. Sakurai for his kindness in taking the photographs of absorption spectra.

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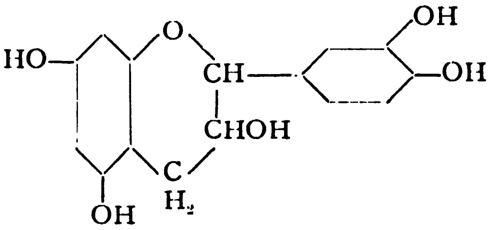
# ON TEA TANNIN ISOLATED FROM GREEN TEA.

By

Michiyo Tsujimura.

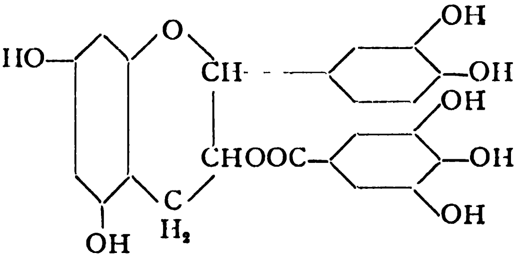
(Received September 8 th., 1930).

The author(1) has recently isolated Tea catechin from green tea and proposed the following formula as its constitution,



Tea catechin ($C_{14}H_{14}O_6$) m p. 237~238°, $[\alpha]_D^{25} = -69°$.

Continuing the studies on tannin substance in green tea, the author has now succeeded in isolating a tannin, which is most probably the gallic acid ester of tea catechin, having the following formula:



Tea tannin ($C_{22}H_{18}O_{10}$).

The author gave it the name "Tea tannin". Tea tannin is an amorphous powder, easily soluble in water, and its aqueous solution has an acid reaction and an astringent taste. When pure, it is nearly colourless, but it is gradually oxidized in the air, to a reddish brown mass. On being boiled with 5% sulphuric acid, it gives gallic acid and a reddish brown substance which yields phloroglucinol, when heated with 50% potash, just in the same manner as in the case of tea catechin. Tea catechin could not be detected among the hydrolytic products of tea tannin, because the later is easily changed to a reddish brown substance by heating with sulphuric acid. The other properties of tea tannin resemble to those of catechin and gallotannic acid. As tea tannin does not give the colour reaction with metallic magnesium and hydrochloric acid, no presence of flavon nucleus can be proved, The result of the analysis of tea tannin agrees with the formula $C_{22}H_{18}O_{10}$ and that of the acetyl derivative with $C_{36}H_{32}O_{17}$ or with $C_{22}H_{11}O_{10}(COCH_3)_7$. The molecular weight of the acetyl derivative was found to be 755 or 809, while the theory raquires 736.

The formation of tea tannin from tea catechin and gallic acid may be illustrated as follows;

$$\underset{\text{Tea catechin}}{C_{15}H_{14}O_6} + \underset{\text{Gallic acid}}{C_7H_6O_5} - H_2O = \underset{\text{Tea tannin}}{C_{22}H_{18}O_{10}}$$

The specific rotation of tea tannin was found to be

$$[\alpha]_D^{23} = -162.5°,$$

and that of acetyl derivative,

$$[\alpha]_D^{23} = -100°.$$

The optical activity must be due to the presence of two asymmetrical carbon atoms in tea tannin, as is shown in the structural formula given above. The tea tannin isolated by the present author closely resembles to the tannin isolated by Deuss⁽²⁾ in 1923. He gave the formulas $C_{20}H_{20}O_9$ and $C_{36}H_{36}O_{17}$ for his tannin and its acetyl derivative respectively. According to him this tannin has at least one CO-and eight OH-groups in its molecule, but no COOH-group. No detail was, however given by him whether phloroglucinol is found by the decompotion of his tannin, so it requires further investigation of his tannin, so it requires further investigation to undertsand the nature of the substance. Quite recently, Ryo Yamamoto and his co-workers⁽³⁾ isolated tea catechin and a tannin from fresh tea leaves of Formosa. The latter substance is stated to have the formula $C_{17}H_{16}O_9$ and to give blue colour with ferric chloride, but apparently it is different from tea tannin.

## EXPERIMENTAL.

### (1) The Isolation of Tea Tannin from Green Tea.

The green tea was finely powdered and extracted at ordinary temperature with ethyl acetate containing 10% water. The extract was evaporated and the residue was taken up in water. When the aqueous solution was treated with a few drops of neutral lead acetate, a thick precipitate was formed, which was filtered off, and the clear filtrate was treated with so much neutral lead acetate as no more precipitate was formed. The yellowish white precipitate thus produced was collected by centrifugal process, washed with water, and decomposed with 10% sulphuric acid. The lead sulphate then was separated by centrifugal machine and filtered, and the reddich yellow filtrate thus obtained was now repeatedly shaken with ethyl acetate. The united ethyl acetate solution was evaporated, the residue was dissolved in water and shaken with ether. The etherial solution, after being washed with water, was evaporated and the residue again dissolved in ether and after being decolorized with a little animal charcoal, evaporated on a water bath at low tamperature. The dried residue thus obtained was then extracted in a Soxhlet apparatus with chloroform ; the insoluble portion was dissolved in alcohol, filtered and evaporated. The residue was again dissolved in ether, decolorized with animal charcoal and washed with water. After repeating this operation, the etherial extract was evaporated, once more dissolved in ether and evaporated on a water-bath at low temperature,

### (2) Important Properties of Tea Tannin.

The freshly prepared tea tannin is nearly colourless amorphous powder which gradually oxidizes in the air, to a reddish brown mass. It is soluble in water, alcohol, ether, ethyl acetate, aceton and glacial acetic acid, but insoluble in chloroform or benzene. The aqueous solution has an acid reaction and an astringent taste, and gives a blue coloration or blue precipitate with ferric chloride solution. It gives also the characteristic phloroglucinol reaction with pine shavings, moistened with concentrated hydrochloric acid. The aqueous solution gives, further, a yellow precipitate with bromine water, a white precipitate with gelatine, a reddish purple coloration with lime water, and a yellowish white precipitate with lead acetate.

For comparison, the chief reactions of tea tannin, catechin, gallic acid and gallotannic acid are shown in the following table :—

| | Tea tannin | Catechin | Gallic acid | Gallotannic acid |
|---|---|---|---|---|
| Taste | astringent | astringent | —— | astringent |
| $FeCl_3$ | blue | green | blue | blue |
| Bromine water | yellow ppt. | yellow ppt. | no ppt. | no ppt. |
| Lime water | reddish purple | red | blue→red | grey |
| Phloroglucinol reaction | positive | positive | negative | negative |
| Gelathine | white ppt. | no ppt. | no ppt. | white ppt. |
| Heating with dil. acid | reddish brown ppt. | reddish brown ppt. | no ppt. | no ppt. |

### (3) Analysis of Tea Tannin.

| | Sample | $CO_2$ | $H_2O$ | C % | H % |
|---|---|---|---|---|---|
| I. | 3.246 mg. | 6.595 mg. | 1.583 mg. | 55.57 | 5.42 |
| II. | 3.672 | 7.487 | 1.709 | 55.61 | 5.17 |
| III. | 3.255 | 6.601 | 1.543 | 55.31 | 5.23 |
| | Average | | | 55.49 | 5.29 |
| | calc. for | $C_{22}H_{18}O_{10}+2H_2O$ | | 55.23 | 4.60 |

### (4) Specific Rotation of Tea Tannin.

$$[\alpha]_D = \pm \frac{\alpha \times 100}{c \times l}$$

$\alpha$: observed rotation $= -3.25°$

$c$: concentration $= 2\%$ in $94\%$ ethyl alcohol

$l$: length of tube in decimeter $= 1$

$$\cdot \quad [\alpha]_D^{23} = -\frac{3.25 \times 100}{2 \times 1} = -162.5°$$

### (5) Decomposition of Tea Tannin with 5% Sulphuric Acid.

[1] Gallic acid :— When tea tannin was boiled with 5% sulphuric acid for two hours, a reddish brown precipitate was formed. By sharking the latter with ether and evaporating the etherial extract, gallic acid was obtained in crystalline state, which gave blue colour with $FeCl_3$ and melted at 237°. Mixed with pure gallic acid, no depression of melting point was observed.

Analysis of Gallic Acid.

| Sample | $CO_2$ | $H_2O$ | C% | H% |
|---|---|---|---|---|
| 2.958 mg. | 4.847 mg. | 1.186 mg. | 44.68 | 4.46 |
| calc. for | $C_6H_2(OH)_3CO_2H+H_2O$ | | 44.67 | 4.29 |

[2] Phloroglucinol :— After the reddish brown precipitate, obtained as above, was heated with 50% KOH at 180° for half an hour, it was cooled, diluted with water, neutralized with sulphuric acid and then extracted with ether. The etherial solution gave crystalline plates on evaporation, which gave all characteristic reactions of phloroglucinol. The purified colourless crystals melted at 213° and the mixture with pure phloroglucinol showed no depression of melting point.

[3] Glucose :— The mother liquor of the reddish brown precipitate was neutralized with barium carbonate, concentrated on a water bath, and thus tested for glucose: no phenylosazone was formed. Glucose was absent.

### (6) Acetylation of Tea Tannin.

The acetylation was carried out in the following two ways.

[1] 0.5 Gram tea tannin was dissolved in 5 c.c. acetic acid anhydride, cooled and treated with one drop of concentrated sulphuric acid; the reaction started immediately. After several hours, the reacting mixture was poured into water, and the precipitate formed thereby was collected, washed with water and dried on a porous tile. For purification it was dissolved it hot alcohol and filtered while hot. On cooling, a white amorphous substance

separated out, *and it was purified from the methyl* alcohol solution.

[2] 0.5 Gram tea tannin was dissolved in acetic acid anhydride; 1.5g. sodium acetate anhydride was added, and heated on a water-bath under reflex cooler for five hours; the reaction mixture was poured into water and the precipitate formed was treated as above.

## Analysis of the Acetylated Tea Tannin.

| | Sample (1) | $CO_2$ | $H_2O$ | C% | H% |
|---|---|---|---|---|---|
| I. | 3.565 mg. | 7.510 mg. | 1.566 mg. | 57.45 | 4.88 |
| II. | 3.264 | 6.853 | 1.382 | 57.26 | 4.70 |
| | Average | | | 57.36 | 4.79 |
| | calc. for | $C_{22}H_{11}O_{10}(COCH_3)_7+H_2O$ | | 57.29 | 4.51 |

| | Sample (2) | $CO_2$ | $H_2O$ | C% | H% |
|---|---|---|---|---|---|
| I. | 3.961 mg. | 8.408 mg. | 1.624 mg. | 57.89 | 4.56 |
| II. | 3.183 | 6.712 | 1.302 | 57.51 | 4.55 |
| | Average | | | 57.7 | 4.56 |
| | calc. for | $C_{22}H_{11}O_{10}(COCH_3)_7+\frac{1}{2}H_2O$ | | 57.98 | 4.42 |

## (7) Determination of Acetyl Value.

[1] The determination was carried out by Freudenberg's method.(4)

| Sample | | N/5 NaOH | $CH_3CO$% |
|---|---|---|---|
| I. | 0.1200 g. | 5.3 c.c. | 37.98 |
| II. | 0.1063 | 4.7 ″ | 38.42 |

[2] The same determination was repeated with Kögl and Postwsky's method,(5) using alcoholic potash for saponification.

| Sample | | N/70 NaOH | $CH_3CO$% |
|---|---|---|---|
| I. | 11.3 mg. | 7.3 c.c. | 39.49 |
| II. | 12.5 ″ | 8.1 ″ | 39.79 |
| | calc. for | $C_{22}H_{11}O_{10}(CH_3CO)_7+\frac{1}{2}H_2O$ | 40.40 |

## (8) Specific Rotation of the Acetyl Derivative.

$$[\alpha]_D=\pm\frac{\alpha\times 100}{c\times l}$$

$\alpha=-1^\circ$

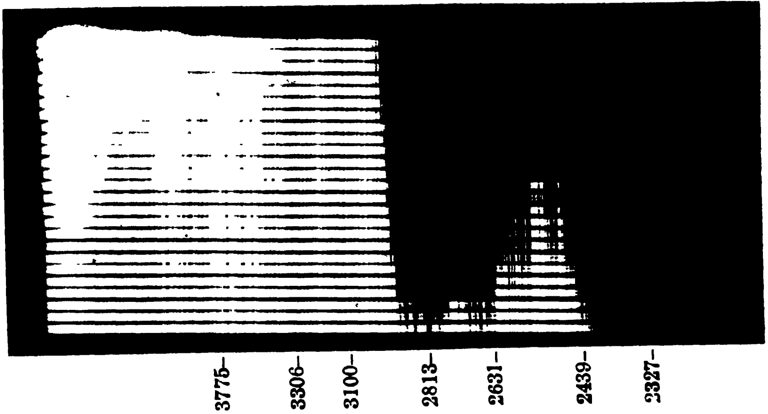
$c=1\%$ in benzene solution

$l=1$ d.m.

$$[\alpha]_D^{23}=-\frac{1\times 100}{1\times 1}$$

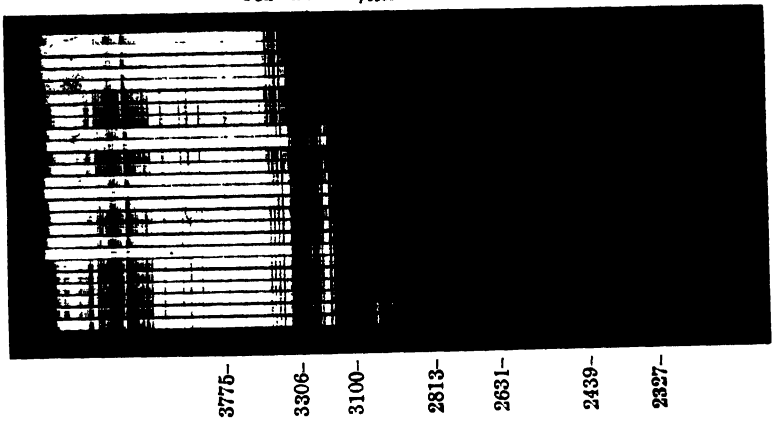
## (9) Molecular Weight.

The determination was carried out by Rust's camphor method.(6)



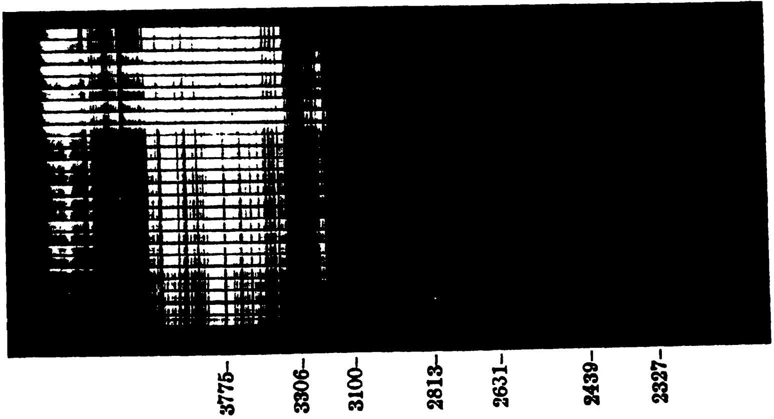
Tea catechin $^1/_{1000}$ mol. alc. sol.

Fig. 1.



Tea tannin $^1/_{5000}$ mol. alc. sol.

Fig. 2.



Gallic acid $^1/_{5000}$ mol. alc. sol.

Fig. 3.

$$m=\frac{c\times w\times 100}{dW}$$

| $m$: | mol. wt. | I. | II. |
|---|---|---|---|
| $c$: | const. | 400 | |
| $w$: | subst. mg. | 8.2 | 20.7 |
| $d$: | depress. of m.p. | 5° | 4° |
| $W$: | solvent mg. | 81 | 273.3 |

$$\text{I.}\quad m=\frac{400\times 8.2\times 100}{5\times 81.0}=809$$

$$\text{II.}\quad m=\frac{400\times 20.7\times 100}{4\times 273.3}=755$$

calc. for $C_{23}H_{11}O_{10}(COCH_3)_7$ 736

The author expresses sincere thanks to Prof. U. Suzuki for his kind direction throughout this work.

(1) Literature loc. cit.
(2) Deuss: Rec. trav. chim., **42**, 496, 1053, 1923
(3) Yamamoto: J. Agr. chem. Soc. Japan, **6**, 564, 1930
(4) K. Freudenberg: Ann., **433**, 230, 1923
(5) Kögl and Postwsky: Ann., **440**, 34, 1924
(6) Karl Rust: Ber., **55**, 1051, 1922

# UEBER DIE OBERFLÄCHENAKTIVITÄT UND DIE ADSORBIERBARKEIT VON AMINOSÄUREN.

## II. MITTEILUNG.

von

TAKEO ITO.

*(Aus dem Agrikulturchemischen Laboratorium in Landwirtschaftlicher Hochschule, Morioka, Japan.)*

(Eingegangen am 12 September 1930.)

In der vorhergehenden Mitteilung (Ito diese Zeitschr., **6**, 13~16, 1930.) wurde u.a. gezeigt, dass eine homologe Reihe von Aminosäuren——von Glykokoll bis Leucin——im grossen und ganzen entsprechend der Traubeschen Regel oberflächenaktiv und adsorbierbar sind. Von den genannten Aminosäuren sind, indessen, Valin und Leucin Isoverbindungen, während die andere normale Verbindungen sind. Es schien mir daher wunschenwert, die erwähnte Ergebnisse durch weitere Versuche mit den entsprechenden normalen Verbindungen zu ergänzen. Aus diesem Grunde wurde in der vorliegenden Untersuchung bearbeitet mit Norleucin ($\alpha$–Amino–n–Capronsäure) und $\alpha$–Amino–n–Valeriansäure. Ferner wurde Versuche an Prolin, welches ähnlich wie Valin bzw. dessen Isomer zusammengesetzt ist, vorgenommen.

Die zur Oberflächenspannungsmessung sowie für die Adsorptionsversuche angewandten Methoden waren ganz ähnlich wie bei der vorigen Arbeit (Lot. cit.). Nur bei Prolin hatte man zur Konzentrationsbestimmung seiner Lösung nach Kjeldahl zu arbeiten, da man hier bekanntlich nicht die Van Slykesche Methode anwenden kann.

Die Resultate sind in den folgenden zwei Figuren graphisch zusammengestellt.

In Fig. 1 sind die $\sigma$–C–Kurven von den drei angeführten Aminosäuren wiedergegeben, gleichzeitig sind die des Valins und des Leucins mit eingezeichnet. Als Ordinate ist die relative Oberflächenspannung $\sigma$ ($\sigma_{H_2O}=1$) zugeordnet, als Abzisse die Konzentration C in Mol im Liter. Es ist ersichtlich, dass Norleucin und $\alpha$–Amino–n–Valeriansäure merklich oberflächenaktiver sind als die entsprechenden Isoverbindungen.

Bei der ähnlichen Zusammensetzung ist Prolin ($\alpha$–Pyrrolidincarbonsäure) auffallend weniger oberflächenaktiv als Valin bzw. dessen Isomer, was offenbar auf die Konstitutionsverschiedenheit zurückzuführen ist.

In Fig. 2 finden sich die Adsorptionsisothermen (Adsorbens : dieselbe Knochenkohle wie bei der schon mehrfach erwahnten Arbeit.). Als Ordinaten sind die adsorbierten Aminosäuremengen in Millimol pro g Kohle aufgetragen, als Abzissen die Gleichgewichtskonzentrationen C in Mol im Liter.

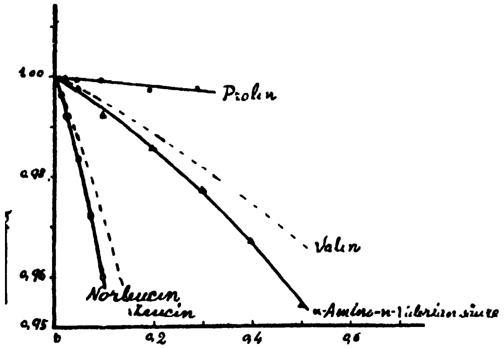


Fig. 1.
σ-c-Kurven von wäßrigen Aminosäurelösungen

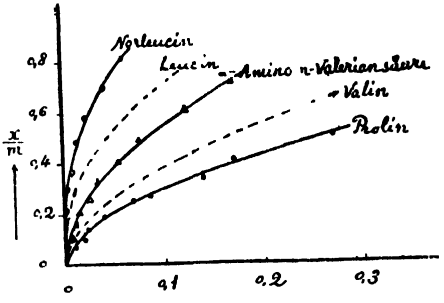


Fig. 2
Adsorptionsisothermen von wäßrigen Aminosäurelösungen

Vergleicht man Fig. 2 mit Fig. 1, so erkennt man wieder einen deutlichen Parallelismus zwischen der Oberflachenaktivitat und der Adsorptionsfähigkeit von den hier in Betracht kommenden Aminosäuren.

# CHEMISCHE UNTERSUCHUNGEN ÜBER DAS GLUKOMANNAN AUS "KONJAK"

## I. MITTEILUNG. DARSTELLUNG DES GLUKOMANNANS.

von

KITSUJI NISHIDA und HIDEO HASHIMA.

(Eingegangen am 3 October 1930)

Nach den verschiedenen Mitteilungen liefert also das Konjak-Mannan nach seinen Untersuchungen bei der Hydrolyse drei Zuckerarten, Glukose, Mannose und Fruktose in verschiedenen Verhaltnissen, nämlich von 1:2:0, 2:5:0 und 2:3:1. Diese Ergebnisse wurden zur Ursache der Annahme für eine Uneinheitlichkeit in der Zusammensetzung des Mannan in den Händen gehabt.

Mannan kann durch kochendes Wasser oder durch Enzyme verflüssigt und durch Alkohol als Aufschlämmung ausgefällt werden, oder durch Enzyme verflüssigt und durch Alkohol als Aufschlämmung ausgefällt werden, aber es enthält dann noch als Verunreinigungen, z. B. Inulin, Pektinstoffe, Galaktan, Fruktan, Xylan und Araban u. a. M.

So hergestelltes Mannan ist durchaus kein reines Produkt, es musste daher von diesen Begleitstoffen isoliert werden. Es ist dennach also vor allen Dingen wichtig, zur Gewinnung grösserer Mengen von Mannan in reinem Zustande ein Verfahren zu seiner Reinigung auszuarbeiten, insbesondere ein Verfahren des Abbaus durchzuführen und auch in der Frage des Verhältnisse der hydrolysierten Zuckermenge ordnungsgemäss vorzugehen.

Karrer und seine Mitarbeiter fanden, dass das Galaktomannan sich in heissem Wasser, leichter noch in verdünnten Laugen auflöst und durch Fehlinglösung daraus als Kupferverbindung wie Xylan nach Salkowski gefällt werden kann. Setzt man dem pulverisierten Konjakmannan genügend viel Wasser unter Umrühren zu, so erhält man das Produkt in kolloidaler Form. Durch Erhitzung mit Wasser unter Druck (1~1.5 Atm.) im Autoklaven wird das Konjak-mannan verflüssigt und man gewinnt dann durch Absaugen ein klares, hellgelbes Filtrat, aus dem durch Fehlingsche Lösung das schön blau gefärbte, voluminöse Kupfermannan ausgefällt wird. Mit alkoholischer Salzsäure zerlegt man diese Aufschlämmung, filtriert das erzeugte Glukomannan ab, wäscht es mit Alkohol und Aether und trocknet es im Vakuum auf Phosphorsäureanhydrid. Das Produkt ist eine feinpulverige, reinweisse Masse, die mit Jod nicht blau färbt und Fehlingsche Lösung nicht mehr reduziert.

Die Ausbeute an Glukomannan und dessen Aschengehalt sind sehr schwankend:

| Nr. | Ausbeute | Aschengehalt |
|---|---|---|
| 1 | 47.41% | 0.40% |
| 2 | 43.62% | 0.28% |
| 3 | 67.72% | 0.10% |
| 4 | 65.59% | 0.19% |
| 5 | 64.85% | 0.45% |
| 6 | 62.65% | 0.32% |

Das durch geschriebene Verfahren gewonnene Glukomannan besteht aus Mannose und Glukose, während die Reaktion auf Fruktose negativ ausfiel. Besonders interessant ist hier, dass das Glukomann aus Konjakpulver durch das Auskochen mit Wasser unter Druck im Autoklaven nicht in siner urspringlichen, sondern in einer bereits chemisch veränderten Form erhalten wird, was äusserlich daran zu erkennen ist, dass die Bestandteile des ursprünglichen Konjakmannans Mannose, Glukose und dazu Fruktose oder nur Mannose und Glukose sind. Es ist also eine Verflüssigung durch Enzyme und Bakterien erforderlich, um Ausblicke auf dieses Ziel zu erreichen.

Hierzu verwendet man 10%ige Pankreatin-Lösung mit Toluol, welche dem Konjakpulver zugefügt werden und mit welchen es im Thermostaten auf 35°C erwärmt und in zwei Wochen verflüssigt wird. Die kolloidale Flüssigkeit wurde wie vorher behandelt. Nach dem Trocken ergib sich ebenfalls ein weisses, lockeres Pulver. Die Ausbeute beträgt 57%, der Aschengehalt 0.47%. Darin sehen wir eine weitere, vollkommene Analogie zwischen diesen durch beide Verfahren gewonnene Glukomannanarten.

## II. MITTEILUNG. ZUSAMMENSETZUNG DES GLUKOMANNANS DURCH DIE HYDROLYSEN.

(Eingegangen am 14 October 1930.).

Wesentlich grösseres Intersse kommt nun der Zusammensetzung des durch A-bezw. B-Verfahren hergestellten Mannans zu, dem Glukomann oder Fruktoglukomannan, und weiter der Frage, ob sich darin Mannose, Glukose und auch Fruktose und in welchen Verhältnisse vorfinden. Ein geeigneter Weg zur Aufklärung dieser Fragen schien uns nun die quantitatv durchgefürte Hydrolyse zu sein. Zum Verzuckern der höheren Kohlenhydrate sind von verschiedenen Forschern verschiedene Reagentien gesucht worden und als geeignetes Mittel dienen hochkonzentrierte Salzsäure und verdünnte Salz und Schwefelsäure.

Zur Hydrolyse des Glykomannans verwendeten wir Salzsäure in 3 verschiedenen hohen Konzentration, nämlich vom spez. Gew. 1.21, 1.223 und 1.23 in der Kälte. Aus diesen Versuchen geht also hervor, dass die Verzuckerung des Glukomannans in 24 Stunden ihrer Ende erreicht und schwankende Zuckermengen, nämlich zwischen 102~108% vom Gewicht des Glukomannans (theoritisch 111.1%) ergibt.

Um die Verzuckerung mittels hochkonzentrierter Salzsäure durch die Hydrolyse mit verdünnter Salz-und Schwefelsäure vergleichen zu können, haben wir das Glukomannan durch Erhitzen mit 10%igen, 5%igen und 2%igen Lösungen beider Säuren aufgespalten. In einer Reihe von Versuche zeigte es sich, dass der Aufschluss von Glukomannan durch verdünnte Schwefelsäure sehr langsam verläuft und es erhellt, dass man durch Hydrolyse mit 2%iger Salzsäure während 25 Stunden im Wasserbade die beste Zuckerausbeute erzielt.

Ueber den genauen Sitz der Fruktose wurde früher von Miyake berichtet, doch lässt sich über dieses Glukomannan nichts sicheres ansagen. Der Nachweis der Fruktose fiel in den hier ausgeführten Versuchen nach Seliwanoff mittels der Resorcinprobe negativ aus, dagegen wurde nach Pinoff mit Ammoniummolybdatlösung eine geringe Blaufärbung beobachtet. Wahrscheinlich aber handelte . es sich im letzten Falle nun Glukose, da die Intensität der Färbung genau so stark war, als wenn die Lösung nur 0.03 gr. Fruktose enthealten hätte. Am besten hat sich bei Abwesenheit von Fruktose die Bestimmung von Aldose nach Kolthoff bewährt. Aus diesen Versuchen geht hervor, dass man den gesamten hydrolysierten Zucker als Aldose ausgezeichnet jodmetrisch bestimmen kann und dass die Lösung keine Fruktose enthält. Was die Bestandteile des durch das A- bezw. B-Verfahren hergestellten Glukomannans angeht, so sehen wir, dass das Glukomannan bei der Darstellung durch kochendes Wasser unter Druck, gleich wie das Enzyme verflüssigte Glukomannan, so gut wie keine Fruktose enthält.

Da bei der Verzuckerung als Abbauprodukt Mannose und Glukose zu erwarten sind, war es von Wichtigkeit, deren Verhalten in Lösung zu beobachten. Zu diesen Zuwecke war Mannosebestimmung mit Phenylhydrazin und die Feststellung des Drehungsvermögens erforderlich. Die durch Feststellung des Drehungsvermögens bei konz. Säurehydrolyse gewonnenen Ergebnisse stimmten nicht so genau überein, dass wir die Behauptung, das Glukomannan sei aus 2 Mol Mannose und 1 Mol Glukose aufgebaut, aufrecht erhalten könnten. Hier seien die Fälle, bei den Vergleichsproben in denen Glukomannan mit verdünnter Säure verzuckert wurden berücksichtigt; bei solchen Proben entspricht die Genauigkeit der polarisierenden Kraft etwa der Genauigkeit der Zuckerverhältniszahlen. Aus diesen Zahlen ist nun zunächst in Uebereinstimmung mit der Angabe Mayeda's zu folgern, dass das Glukomannan aus 2 Mol Mannose and 1 Mol Glukose aufgebaut ist.

Nach mannigfaltigen Versuchen mit hochkonzentriertem und verdünntem Säureabbau die Zuckerverhaltnisse in bezug auf die Menge durch Mannosephenylhydrazonbestimmung wurde befriedigenden Ergebnisse angeführt. Aus dieser Reihe von Vorversuchen ergibt sich, dass das Konjakmannan bei der Hydrolyse 1 Mol Glucose und 1 Mol Mannose lieferte, danach es also als ein Glukomannan aufzufassen wäre. Diese Verhältniszahlen sind, da 1.2 Mol Phenylhydrazin theoretisch genügen, praktisch aber viel weniger hinzugesetzt wurde, nicht brauchbar. (Bei diesen Fällen wurden 1.2 Mol Phenylhydrazin im Becher abgewogen and darauf die 3 fache Menge Eisessig hinzugefügt. Diese Gemisch wurde wieder im Erlenmyerkolben auf die Lösung übergegossen und die Reaktion abgewartet. Hierbei wurde das Phenylhydrazin nicht so gut gemischt und nach dem Abgiessen blieben geringe Mengen in dem Becher zurück). In Hydrolyse soll versucht werden, eine zahlmässige Schlussfolgerung dieser Angabe herzuleiten, wobei die Ermittelung mit 1.5 Mol, 3 Mol und 5 Mol Phenylhydrazinzusatz aufgeführt wurde.

Bei der Betrachtung der Analyseergebnisse fällt zunächst auf, dass die Fehlerquellen und die Ausarbeitung genügend fehlerfreie Bestimmungsverfahen gelöst, und ein deutlisches Bild der Verhältniszahlen erhalten werden kann. Das Polarisationsverfahren bei verdünnter Säurehydrolyse zeigt fast völlige Uebereinstimmung mit dem Phenylhydrazonsverfahren. Danach ist das Glukomannan von Konjak ein polymeres Kohlenhydrat, das aus 2 Mol Mannose und 1 Mol Glukose besteht, d. h. es ist Glukomannan.

(Forstchemische Institut, Kaiserl. Kisuhu Universität).

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# UNTERSUCHUNG ÜBER DAS ÄTHERISCHE OEL AUS PODOCARPUS MACROPHYLLA, DON.

## I. MITTEILUNG. DER BESTANDTEIL DES ÄTHERISCHEN OEL AUS PODOCARPUS MACROPHYLLA, DON

von

Kitsuji Nishida und Hidetake Uota.

(Eingegangen am 26 October 1930).

Podocarpus macrophylla, Don. ist im südlichen Japan, in Formosa und im südlichen China eine etwas häufige Ercheinung. Bei der Destillation des Astes und des Blattes wurde 0.049% ätherisches Oel erhalten. Die Konstanten des nicht unangehem riechenden Oels waren folgende: $d_4^{15}$ 0.9350, $[\alpha]_D^{20}$ $-7.56°$, $n_D^{20}$ 1.50819, Säurezahl eine Spur, Esterzahl nach Acethylierung 26.1.

Das Oel wurde im Vakuum destilliert und die folgenden Fraktionen aufgefangen:

| Fraktion | Drück | Temperatur | % | Farbe | $d_4^{15}$ | $n_D^{20}$ | $[\alpha]_D^{20}$ |
|---|---|---|---|---|---|---|---|
| I | 6 mm | 42\~44° | 4.00 | farblose | 0.8555 | 1.46847 | +28°02 |
| II | 5 〃 | 46\~48° | 4.13 | 〃 | 0.8513 | 1.47131 | +46°40 |
| III | 5 〃 | 56\~60° | 2.53 | hellgelb | 0.8572 | 1.47040 | +57°58 |
| IV | 3 〃 | 68\~70° | 0.70 | 〃 | —— | 1.48613 | +27°60 |
| V | 3 〃 | 90\~94° | 1.07 | 〃 | —— | 1.49342 | + 1°40 |
| VI | 3 〃 | 104\~105° | 1.31 | 〃 | —— | 1.49492 | − 5°72 |
| VII | 4 〃 | 107\~110° | 9.05 | 〃 | 0.9110 | 1.49596 | − 7°60 |
| VIII | 4 〃 | 113\~115° | 2.93 | 〃 | 0.9148 | 1.40417 | − 4°92 |
| IX | 3.5 〃 | 110\~112° | 2.23 | 〃 | 0.9170 | 1.50096 | − 5°16 |
| X | 3.5 〃 | 123\~125° | 3.13 | 〃 | 0.9253 | 1.50591 | − 9°20 |
| XI | 3.5 〃 | 138\~140° | 5.88 | 〃 | 0.9400 | 1.51062 | − 9°23 |
| XII | 3 〃 | 153\~169° | 47,33 | 〃 | 0.9710 | 1.52201 | −13°32 |
| XIII | 3 〃 | 173\~177° | 9.34 | 〃 | 0.9873 | 1.52738 | −40°92 |
| Rückstand | | | | rotbraun | | | |

Aus verseiftem Oel.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| I′ | 4.5 mm | 39~40°5 | 7.10 | farblose | 0.8523 | 1.471016 | +33°40 |
| II′ | 5 ″ | 45~47° | 3.34 | ″ | 0. | 1.472094 | +54°64 |
| III′ | 5 ″ | 99~101° | 5.74 | hellgelb | 0.9040 | 1.493420 | − 1°00 |
| IV′ | 4 ″ | 104~106° | 5.83 | ″ | 0.9099 | 1.498384 | − 9°40 |
| V′ | 4.5 ″ | 109~120° | 8.08 | ″ | 0.9150 | 1.501606 | −10°60 |
| VI′ | 3 ″ | 138~140° | 10.24 | ″ | 0.9483 | 1.514000 | −11°52 |
| VII′ | 2.5 ″ | 156~157° | 17.45 | ″ | 0.9687 | 1.520818 | −20°60 |
| VIII′ | 2.5 ″ | 157~158° | 11.09 | ″ | 0.9715 | 1.522624 | −24°00 |
| IX′ | 2.2 ″ | 162~164° | 13.93 | dünntesten | 0.9764 | 1.523815 | −32°40 |
| X′ | 2.2 ″ | 164~165° | 11.10 | hellgelb | 0.9828 | —— | —— |
| XI′ | Rückstand | | 1.03 | dünkelbraun | —— | —— | —— |

Die niedrigst siedenden Anteile des Oels (I, II und I′ Fraktion $[\alpha]_D^{20}$ (+)46°~(+)28°) gab mit Amylnitrit und Salzsäure ein Nitrosochlorid (Smp. 103°C). Es war auch möglich, α-Pinennitrosochlorid durch Umsetzung mit Piperidin zu Pinennitrolpiperidin vom Smp. 117~118°C nachzuweisen. Bei der Prüfung der II und III Fraktion auf Camphen mit Eisessig-Schwefelsäure wurde Isobornylacetat erhalten, das bei der Verseifung Isoborneol ergab. Fenchen war in der I Fraktion nicht enthalten. β-Pinen liess sich in dem Anteil von Smp. 56~70°C (3~5 mm) aus der III und IV Fraktion mit Sicherheit nachweisen. Es entstanden bei der Oxydation mit alkalischen Permanganat gläzende Blättchen eines nopinsaures Natrium, die leicht Nopinsäure (Smp. 121−122°C) darstellen. V und III′ Fraktion gaben nur eine Abscheidung eines bei 118°C schmelzenden Cadinenchlorhydrat. Dagegen waren diese Fraktionen nicht caryophyllen und sylvestrenhaltig. Zwei neue Dipentenen aus XI~XIII and VI~X′ Fraktion wurden in II Mitteilung erklärt.

(Forstchem. Institut, Kaiserl. Kiushu Universität).

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# ON GLUCONIC FERMENTATION PART I.*

## ON BACTERIUM HOSHIGAKI VAR ROSEA NOV. SPEC.

By

TÉIZO TAKAHASHI and TOSHINOBU ASAI.

(Received 18th., Nobember, 1930.)

Boutroux's(1) memoir on gluconic acid formation as a fermentation product

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* This was already published in "Nihon Nōgei Kwagaku Kwaishi" Vol. VI. Vo. 3. March. 1930. (Japanese language).

offered in 1880, cast us very interesting and important problem; although the occurrence of gluconic acid in the product of his lactic acid fermentation was quite beyond the scope of his expectation; viz. the lactic acid. Later, Brown(2) confirmed more satisfactory in bacteriological point of view the formation of this acid by his Bact. aceti and xylinum. Bact. Kützingianum and Bact. Pasteurianum behave in the same way after Seifert(3). Almost all species of Henneberg's(4) acetic bacteria and in likewise Termobacterium aceti produce gluconic acid from glucose after Zeidler(5). Hoyer's(6) two varieties of Bact. rancens viz. var. Zythi and var muciparum and Acetobacter melanogenum after Beijerinck(7), as well as Miyaji's(8) Bact. acetigenum var. Bact. rancens var. and Bact. vini acetati var. and a variety of Bact. industrium of Yagi and Hashitani(9) belong to the same category of acetic bacteria.

Recently, S. Hermann(10) isolated from Japanese Kombucha (Algae-tea) special bacteria named Bact. gluconicum, which producing an abundant quantity of gluconic acid. The percentage of the acid formed from glucose attained in his case to about 80, excelling all instances prior to him.

R. Falk and S. R. Kapurs'(11) observation gave some light upon the nature of the gluconic fermentation of mould. They concluded that Asp. niger, Asp. cinnamomens, Asp. fuscus and other fungi causing citric fermentation produce gluconic acid. About Asp. niger K. Bernhaner(12) mentions the same fact, and C. Wehmer(13) found the same acid in the fermentation product of Asp. fumaricus. K. Sakaguchi's(14) observation on the varieties of Asp. oryzae confirmed the occurrence of this acid in their products by the great majorities. The same author gave evidence on the production of 50% of the acid stated by Asp. wentii.

The fungus of penicillium group also behave to glucose in the same way, especially Penicillium luteum has been shown to form this acid after O. E. May, Thom and M. B. Church(15). The variety of the said fungus viz., Pen. luteum purpurogenum produce this acid up to 56~57% or even to 64.7% if there were associated special mineral salts to the nutrient medium after O. E. May and H. I. Herrick(16).

In regard to the products of Rhizopus species one of us (T. T)(17) with his co-worker K. Sakaguchi affirmed this acid associated many other organic acids.

Recently, the authors, in the long (about four months) culture of Absidia viz., Ab. spinosa as well as Ab. septata confirmed the occurrence of gluconic acid, especially by the former species amounting to 60% as its yield. The details will be expected to communicate in a short while.

S. Hermann's(18) observation quoted above, stimulated us to undertake in the similar way about "Kombu-cha" as well as to follow research about

"Hoshigaki" or dried persimmon with anticipation that an analogous bacteria should be found from the latter material. Unfortunately, we could not find out any acetic bacteria from the former until now inspite of many samples were examined. On the other hand, from dried persimmon four varieties of acetic bacteria, one lactic bacteria, two mycoderma yeasts and one mould were isolated.

All of these acetic bacteria are quite new species and moreover produce gluconic acid from glucose amounting to 80~100%, especially one of them specifies itself in producing a beautiful red colouring matter when it was cultured in medium associated with $CaCO_3$. In this paper, inter alia, this special one is to be described, the other three species will be published before long.

## Experimental Part.

### *The fermentation products of "Hoshigaki"*

To prepare the fermenting mash, 20 g. of "Hoshigaki" was chopped into small pieces, which were introduced into a sterilised flask containing 200 c.c. of sterilised water. The flask was held in an incubater at 28~33°C during ten days.

In the begining the evolution of the gaseous matter were perceived, thence followed the formation of the film on the surface of the fluid associating the brown deposit at the bottom of it. At the end of incubation there was perceived considerable amount of acid(1), which attained in terms of n/10 NaOH solution up to 12.3c.c. when 10c.c. of the fermented fluid was examined.

By subjecting the fermented fluid to the steam distillation, only a trace of volatile acid were perceived, suggesting the predominancy of some non-volatile acid in it.

The latter acid was isolated in the form of salt as crystallised mass, seeming like cauliflower, from the residual fluid of the steam distillation, provided the neutralisation with $CaCO_3$ and condencing of it. The salt was difficultly soluble in the cold water but easily when hot and insoluble in alcohol.

### *Identification of the acid.*

(1) It gave the characteristic blue colour reaction to β-naphthol(19) in presence of $H_2SO_4$.

(2) Its Ca-salt is easily soluble in hot water and precipitated by alcohol. On standing the solution in dilute (50%) alcohol, there comes force beautiful needle shaped crystalls of Ca-gluconate.

(3) The water of crystallisation of barium salt seems to be just one molecule thus :——

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(1) The acidity of original fluid, in terms of n/10 NaOH solution, was 0.1 c.c. for 10 c c. of it.

| | |
|---|---|
| Subst. taken | 0.5099 g. |
| After drying at 110~115°C | 0.4919 g. |
| Differencc. | 0.0180 g. |

∴ % of $H_2O$ = 3.53

Calc. as one molecule of water 3.30%.

(4) The contents of barium in the salt agree with that of gluconic salt thus :——

| | | | |
|---|---|---|---|
| Subst. taken (water free) | | | 0.4919 g. |
| $BaSO_4$ weighed | 0.2168 g. | ∴ | Ba. 0.1276. |
| | Found. | | 25.92% Ba. |
| | Calcul. | | 26.04% Ba. |

(5) The melting point of its phenylhydrazid was 196~200°C, coinsiding with that given in the literature. The përcentage of nitrogen of the same was :——

| | | | |
|---|---|---|---|
| Subst. taken | 0.5022 g. | N. gained | 0.04934 g. |
| | | Found. | 9.83% N. |
| | | Calcul. | 9.79% N. |

(6) The specific rotatory power was + 5.91° thus :——

Subst. taken (Ca-salt) 1.1325 g. water 10 c c.

Temp. 21°C. l = 1 d. m.

$\alpha = +0.67°$

$$\therefore \ [\alpha]_D^{21} = \frac{\alpha \times 100}{d \times l} = \frac{0.670 \times 100}{11.3225 \times 1} = +5.91°$$

For the reference,

| | |
|---|---|
| One of us (T. T) and Sakaguchi's(20) result. | $[\alpha]_D^{17} = +5.73°$ (C = 3.227) |
| Herzfeld(21) | $[\alpha]_D = +5.8 \sim +5.94°$, (C = Ca 2.) |
| Bertrand(22) | $[\alpha]_D^{20} = +6.13°$, (C = 5.0) |
| Fischer(23) | $[\alpha]_D = +6.66°$ |

## *Bacterium Hoshigaki var rosea. nov. Spec.*

This bacteria was isolated from a fermenting mash prepared as the mixture of chopped "Hoshigaki" and sterile water in flask, which was sterilized prior to the introduction of the materials. It forms acetic acid in alcohol fluid but gluconic acid makes its predominancy in case of the nutrient medium containing sugars and devoid of alcohol. It produces *rose red colour* in medium containing $CaCO_3$ and from this character we propose to call this bacterium, ***Bacterium Hoshigaki var rosea.***

Form and Size :—— Short rods, 1.5~1.8 μ long, about 0.7~0.9 μ thick. Generally single; at most in pairs; often in chains. Non-motile. It has vigorous tendency to form involution forms at room temperature. The pellicle on fluid media has no reaction of sterch or cellulose.

Plate cultures. (Incubated at 26~28°C.)

**Koji-Extract Agar :——** After two days appear as small round granular shining colonies, with elevation at the center, which altered to brown colour. Magnified to 150 times: Smooth border with light brown colour and the center is coloured dark brown with compact granular surface.

Wort Agar:—— Round milky white colonies after two days. The center of them gradually alters to brown, in contrast to yellowish brown periphery.

Glucose Sake' Agar:—— Round, milky white granular colonies with elevation of center and smooth periphery after two days. Colour of center changes from yellowish brown to brown, when periphery acquires yellowish white tint.

"Hoshigaki"-Extract Agar:—— Round, milky white granular colonies after two days. Gradually, the center acquires yellowish brown colour and periphery gray white.

## Agar Stab. (Incubated at 26~28°C.)

| Media. | Remarks. | Form and size of cells. | Involution form. |
|---|---|---|---|
| "Koji"-Ext. Agar. Stab: | Feable growth. Surface growth: Gray white, shining flat growth after two days. Later, acquiring brown colour going to lusterless. Medium at stab mouth changes to brown. | Short red, single or in pairs.<br>0.7~0.9μ×<br>0.7~1.45μ | none. |
| Wort Agar. Stab: | Almost no growth. Surface growth: Flat growth with paste-like luster after two days. Later it aquires from gray white to brown colour. Medium under surface growth acquires dark brown culour | Short rod. | Occurs Vely seldom |
| Glucose Yeast Water Agr. Stab: | Feable growth like entangled hair near the mouth, coloured reddish brown which changes to light brown in deep part. Suface growth: White round growth with pasty luster after two days. Later it becoms flat. | Short rod. | Frequently occurs.<br>6~10μ long. |
| Glucose Sake' Agar. Stab: | Feable growth. Surface growth: Milky white round growth after two days. Later acquires fine light rose colour accompanying the elevation of periphenry. Medium changes somewhat brown. | Short rod, single, pair or in chain.<br>0.5~1.45μ×<br>0.5~1.8μ | None. |
| Bouillon Agar. Stab: | Almost no growth in stab canal. Strface growth: Gray white pasty flat growth. | | |
| Yeast Water Agar. Stab: | Almost no growth in stab canal. Surface growth: Almost transparent irregular growth with luster around the mouth of stab. | | |
| "Hoshigaki" Extract Agar. Stab: | Feable growth chiefly at upper part of stab canal. Surface growth: Gray white flat colony with luster. The central part changed to brown. | Short rod. | |

## Agar Streak. (Incubated at 26~28°C.)

| Media. | Remarks. | Cell form and size. | Involution form. |
|---|---|---|---|
| "Koji"-ext. Agr: | Grayish-white, shining growth, with pasty appearance, showing hairy outgrowths. Later it colours purple brown to brown. The water of condensation is turbid with film and deposit. | Short rod, single or pairs. Rarely in chains. 0.7~0.9μ×0.7~1.45μ. | None. |
| Wort Agar: | Generally same as above, with exception of dense brown colour which degradate to light brown on margin part by this medium. Beside no hairy out-growth appears in this medium | Same as above with size of 0.7~0.9μ ×0.7~1.1μ. | Occurs very seldom with size of 2μ×7μ. |
| Glucose Yeat Water Agar: | Same as above with diffence of wavy periphery. The water of condensation is turbid with light brown deposit. | Same as above with size of 0.5~0·7μ ×0.7~0.9μ. | None. |

| | | |
|---|---|---|
| Glucose Saké Agar: Same as above except the yellowish brown coloured marginal elevation along innoculation streak. The water of condensation is turbid with gray white deposit. | Short rod, single or pairs. Seldom in chains with size of 0.5~0.9μ× 0.5~1.45μ. | None. |
| Bouillon Agar: The growth remains limited to the inoculation streak, although it has a pasty consistency. | Short rod, single or pairs, with size of 0.5~0.9μ× 0.5~1.45μ. | Swollen cells with 1.5× 2.0~3.0μ |
| Yeast Water Agar: Feable growth along innoculated streak. The colour is light brown. | Same form and size as above, but chain forms are found. | Swollen cells with size of 3μ×4μ. |
| "Hoshigaki"-Ext. Agar: Pasty growth along inoculation streak. The brown colour is dense at central part in contrast to light brown periphery. | Same form and size as above. | None. |

## The culture in fluid media. (Incubated at 26~28°C).

| Media. | The growth. | Form and size of cells. | Involution form. | Remarks. |
|---|---|---|---|---|
| "Koji"-Extract: | The fluid becomes turbid after two days. Thin film begins to appear after three days, and gradually ascends to the wall of tube. | Short rod single, pairs or in chain with size of 0.9× 0.9~1.45μ. | It occurs seldom as long thread, 1×2~4μ or 1×40μ. | |
| Wort: | The fluid turns turbid after three days, with film on surface. The peposit turns reddish brown after long. | Same as above. 0.7~0.9 × 0.7~1.45μ | Occurs very seldom, 1.2×1.5μ. | The fluid turns red after 5 days. |
| "Hoshigaki"-Extract: | The fluid turns turbid after three days, with very thin film. A trace of bacteria ring was observed. The deposit and bacteria ring change to yellowish brown at the end of the cultivation. | Same as above, but with different sizes as such, 0.9× 1.45~1.8μ. | Occurs very rare. | |
| Hopped Wort: | The film is formed over turbid fluid after two days. It ascend mor or less to the wall of vessel, giving rise the formation of bacteria ring, which changes to brown as deposit alters to same colour. | Same as above, except the size of 0.7~0.9μ×0.7~1.8μ. | Occurs seldom as curved therad. | The fluid turns red colour after five days. |
| Alcohol free Beer: | Turbid after 24 hours, with very thin film. Later, bacteria ring and deposite colours brown. | Same as in wort. | Occurs very rare. | |
| Yeast Water: | Somewhat turbid after two days with coarse bacteria ring. | Same as hopped wort adding chain-form by this medium. | None. | |
| Glucose Yeast Water: | Bacteria ring formed after two days. After three days, film appear under which the fluid turns turbid. The deposit and ring colour brown after long. | Same as in wort. | Thread cells are observed, sttaining to 30μ long. | |
| Bouillon: | The fluid turns somewhat turbid after two days associated with bacteria ring. Film appears after five days. | Short rod, single, pairs or rarely in chains, with the size of 0.9μ× 1.45~1.62.μ | Occurs rarely with the length of 12~20μ | |

| | | | |
|---|---|---|---|
| Alcohol free Glucose Saké: The fluid turns turbid with film over the surface after two days. The deposit and bacterta ring gain brown colour. | Same as in wort. | Almost none. | After four days the fluid gains fine red colour. |
| Glucose Saké: (Alcohol Ca. 4%). Bacteria ring begins to form after two days. The fluid turns somewhat turbid and the film ascends to the wall of vessel. Deposit and bacteria ring colour brown. | Short rod, single with the size of 0.9μ×0.9~1.8μ. | Occurs abundant as long thread with swollen bulb. Some attain to 50μ length | The fluid gains red colour. |
| Beer: (Alcohol Ca. 4%). The fluid turns turbid with thin film which ascends somewhat, after three days. | Same as in yaest water or hopped wort, wanting chain form. | Occurs as thread or swollen thread with size of 1.5~2.0μ×2.0μ. | |
| Alcohol (3%) "Koji"-Ext: Same as in beer. | Same as in glucose yeast-water. | Occurs as short long or swollen thread, attaining to 100μ in the latter case. | |
| Alcohol (3%) Wort: Same as in beer but addition of brown coloured bacteria ring. | Same as in glucose yeast-water, wanting chain form. | Occurs seldom, as curved thread. | |
| Alcohol (3%) "Hoshigaki"-Ext.: Same as in beer. | Same as in alcohol wort. | Occurs as long thread. | |
| Alcohol (3%) Glucose-Yeast-Water: Same as in alcohol wort. | Same as in yeast-water, adding chain form. | Occurs as swollen or curved short thread. | |
| Alcohol (3%) Bouillon: The fluid alters turbid after three days. | | | |
| Führmann's solution: No growth. | | | |
| Beijerinck's solution: No growth. | | | |
| Pasteur's solution: No growth. | | | |
| Janke's solution: No growth. | | | |
| Henneberg's solution: (Asparagin). No growth. | | | |
| Same but pepton: Bacteria ring at the wall of vessel after two days. Turbidity appeared with white spotted film. | | | |
| Same, but Urea: No growth. | | | |
| Henneberg's II. solution: (devoid of alcohol). No growth. | | | |

N. B. Füermann's solution:(24) Am-phosphate 0.2 g., Am-sulphate 0.1 g., Acid K-phosphate 0.1 g., Mg-sulphate 0.1 g., Distil. water 800 c.c. glucose 5%.

Beijerinck's solution :(25). Am-phosphate 0.5 g., KCl 0.1 g., Distil. water 1000 c.c. Alcohol 3%.

Pasteur's solution :(26). Am-phosphate 0.2 g., K-phosphate 0.1 g., Ca-phosphate 0.1 g., Acetic acid glacial 12.5 g., Alcohol 22.5 g., Distil, water 1000 c.c.

Henneberg's II solution :(27). Am-sulphate 0.3 g., Acid K-phosphate 0.3 g., Mg-sulphate 0.2 g., Glucose 5 g., Alcohol 2 g., Distil. water 100 c.c.

Janke's solution :(28). Am-phosphate 1 g., Acid-K-phosphate 0.4 g., Mg-sulphate 0.4 g., Glycerin 5 c.c., Succinic acid 1 g., Distil. water 1000 c.c.

Henneberg's solution :(29). Peptone (or Asparagin, Urea) 10 g., Acid-K-phosphate 1 g., Ca-phosphate 1 g., Mg-sulphate 1 g., Glucose 50 g., Distil. water 1000 c.c.

### *Acid formation from carbohydrates and alcohols.*

(Incubated at 26~28°C during 17 days.)

The medium used was yeast water, containing 2% of alcohol or carbohyhydrate. Control yeast water seeded with bacteria, needed 1.1 c.c. of n/10 NaOH solution to neutralize 10 c.c. of medium after nine days and increased to 1.15 c.c. of the former after seventeen days.

As the result the production of acid was ascertained in the cases of arabinose, glucose, galactose, glycerin, mannit, ethyl alcohol, propyl alcohol, as well as from saccharose, maltose, lactose, dextrin, inulin, glycogen, sorbit, isodulcit, and methyl alcohol although only a trace, but starch and raffinose could not stand for as the source of acid production.

The details are tabulated below :——

| Carbohydrates or alcohols. | Acidity (c.c. of n/10 NaOH. for 10 c c. of the culture). | | Remarks. |
|---|---|---|---|
| | After nine days | After seventeen days. | |
| Arabinose. | 4.5 | 4·9 | |
| Glucose. | 11.1 | 12.1 | |
| Fructose. | 1.45 | 1.5 | Faint growth. |
| Galactose. | 5.0 | 5.0 | |
| Saccharose. | 1.1 | 1.3 | Slight growth. Medium dose not reduce Fehling's solution. |
| Maltose. | 1.2 | 1.3 | Slight growth as shown in saccharose case. |
| Raffinose. | 1.15 | 1.15 | Faint growth. |
| Dextrine. | 1.2 | 1.35 | |
| Starch. | 1.1 | 1.1 | Very feable growth without film formation. |
| Glycogen. | 1.3 | 1.3 | Almost no growth. |
| Inulin. | 1.3 | 1.3 | Faint growth. |
| Glycerin. | 1.8 | 2.0 | Medium reduces Fehling's solution when hot. |

| | | | |
|---|---|---|---|
| **Mannit.** | 2.05 | 1.9 | Do. |
| **Sorbit.** | 1.3 | 1.4 | Do. |
| **Isodulcit.** | 1.0 | 1.3 | Do. |
| **Methyl alcohol.** | 1.3 | 1.3 | |
| **Ethyl alcohol.** | 20.4 | 23.9 | |
| **Prophyl alcohol.** | 20.8 | 22.5 | |

### *Registance against glucose and its optimum concentration for the growth.*

The yeast water(2) containing glucose, varying from 5 to 50%, was used for ascertaining the registant power of this bacillus against glucose and optimum concentration for its growth of the latter.

It was incubated at 26~28°C during twenty days and as the result gained about 40% of glucose as the highest limit of concentration for the growth, in as much as we could not find out any growth in 55% of the same.

The optimum concentration of glucose was 10%, although the highest concentration of gluconic acid gained was 17.27%, which was found in the solution containing 20% of glucose.

The table below shows the details :——

| % of glucose. | After | | | | | Acidity(3) after 20 days, c.c. of n/10 NaOH | Gluco-nic a. %. |
|---|---|---|---|---|---|---|---|
| | 2 days. | 4 days. | 9 days. | 11 days. | 20 days. | | |
| 5.0 | Fluid turbid with yellowish milky white bacteria ring and heavy yellow deposit. | Same as before, but with brown bacteria ring. | | | Deposit altered to brown. | 28.0 | 5.48 |
| 10.0 | Same as above. | Deposite increased. | | | Deposit altered to brown. | 52.8 | 10.34 |
| 20.0 | Little turbid. | Milky white bacteria ring, with deposit. | deposit increased. | | Deposit altered to yellowish brown. | **88.0** | **17.27** |
| 30.0 | —— | Little turbid. | Turbid increased with bacteria ring. | | Turbid added yellowish brown; deposit. | 81.2 | 15.91 |
| 35.0 | —— | —— | —— | Little turbid. | Heavy turbid with milky white bacteria ring and yellow deposite. | 38.2 | 7.48 |
| 40.0 | —— | —— | —— | Little turbid. | Added slight deposit. | 9.2 | 1.80 |
| 45.0 | —— | —— | —— | —— | —— | —— | —— |

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(2) Its original acidity corresponds to 0.6 c.c. of n/10 NaOH solution for 10 c.c.

(3) The acidity shown below was obtained from the difference of the acidity after cultivation and that of the original yeast water.

### *Registance against acetic acid.*

For the ascertion of the registance against acetic acid of this bacillus, beer(4) was taken as original medium and to which acetic acid was added in the desired percentage, varying from 0.5 to 2.5%. The bacillus could not show any growth in beer which added 2.0% of the stated acid. The details are to be seen in the table below :——

| % of acetic acid added. | After | | | | | Acidity shown by c.c. of n/10 NaOH | |
|---|---|---|---|---|---|---|---|
| | 3days | 4days | 8days | 13days | 19days | After growth | Before growth |
| 0 | Fluid turbid, thin film with white ring. | —— | —— | —— | —— | 44.8 | 1.2 |
| 0.5 | No growth. | Little turbid with thin film and ring. | —— | —— | —— | 46.8 | 8.3 |
| 1.0 | No growth. | No growth. | Little turbid with thin film and ring. | —— | —— | 42.8 | 16.6 |
| 1.5 | No growth. | No growth. | No growth. | Very faint turbid with slight ring. | —— | 45.6 | 24.9 |
| 2.0 | No growth. | —— | —— | —— | —— | 46.8 | 38.2 |

### *Registance against ethyl alcohol and the maximum yield of acetic acid.*

To determine the registance against ethyl alcohol of this bacillus, yeast water(5) was used as the medium, adding ethyl alcohol in the desired percentage viz: 1.0~8.0%. All the cultures were incubated at 26~28°C during 20 days. In the medium containing 7% of alcohol, this bacillus could not show any growth and any production of acetic acid. The optimum concentration of ethyl alconol for the production of the acid was 3 vol. %, gaining 1.87% acetic acid.

The table below shows the details :——

| % of alcohol added. | After | | | | | | Acidity(6) gained after 20 days. c c. of n/10NaOH | % of acetic acid. |
|---|---|---|---|---|---|---|---|---|
| | 2days | 3days | 6days | 9days | 12days | 20days | | |
| 0 | Little turbid with very faint film. | —— | —— | —— | Turbid added bacteria ring and yellow white deposit. | As before | 1.3 | —— |

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(4) This beer contained 4 vol. % of alcohol and the acidity as such;— needed 1.2 c.c. of n/10 NaOH solution to neutralize 10 c.c. of the beer.

(5) The acidity of the original medium was 0.6 c.c. n/10 NaOH for 10 c.c. of the medium.

(6) Acidity quoted below is the difference of the acidity of culture and that of the control.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1.0 | As above with addition of yellow powderly deposit. | Film ascend to wall of the vessel. | —— | —— | Heavy turbidity | As before | 21.8 | 1.30 |
| 3.0 | Little turbid with very faint film. | —— | bacteria ring appear. | —— | Turbid increased with slight deposit. | As before but added deposit. | 31.2 | 1.87 |
| 4.0 | As above. | —— | As above. | —— | As above. | As before but added deposit. | 21.4 | 1.28 |
| 5.0 | No growth. | Trace of turbid. | —— | —— | Turbid added the deposit. | As before but added deposit. | 21.4 | 1.28 |
| 6.0 | No growth. | —— | —— | Trace of the turbid | Increased turbid. | Deposit only | 1.5 | Trace. |
| 7.0 | No growth. | —— | —— | —— | Trace of turbid. | Trace of deposit. | —— | —— |
| 8.0 | No growth. | —— | —— | —— | —— | —— | —— | —— |

## *Registance against common salt.*

(Incubated at 26～28°C during 22 days.)

"Koji"-extract(7) (12°B) as culture medium containing the desired concentration of common salt was used for the determination of the registance of this bacillus against common salt, varying from 0.5 to 2.5%. At the end of the cultivation, the limit of the contents of common salt for the growth was found to lie between 1.5～2.0%. In general, common salt in the medium always plays some role for the deterioration of the growth of this bacillus.

The details are tabulated below:——

| % of NaCl added. | After | | | | | Acidity(8) after 22 days:—— c.c. of n/10 NaOH. for 10 c.c. |
|---|---|---|---|---|---|---|
| | 1day | 2days | 4days | 10days | 22days | |
| 0 | Very thin film. | Film ascending with turbid. | As before adding yellow brown ring. | Ring altered to brown. | —— | 46.6 |
| 0.5 | Trace of bacteria ring. | Added trace of turbid. | Added some deposit. | Deposit altered to brown. | —— | 29.0 |
| 1.0 | No growth. | Faint ascending film. | —— | Added milky white ring and trace of brown deposit. | —— | 19.0 |
| 1.5 | No growth. | —— | —— | Faint film, with brown deposit. | —— | 11.0 |
| 2.0 | No growth. | —— | —— | —— | —— | 4.0 |

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(7) The acidity of the medium was 2.2 c.c. of n/10 NaOH for 10 c.c. of it.

(8) Acidity is given from the difference of c.c. of n/10 NaOH for 10 c.c. after 22 days and that of the original solution.

### *Temperature for the growth, acid production and death.*

The optimum temperature for the growth was 30~35°C and at 40~41°C there was no growth although it makes very slow and slight growth at 10~15°C.

The death of the bacillus comes force when it is treated five minutes at 50°C in water.

The optimum temperature for the acid production lies between 26~28°C as it is shown in the following table:— (The culture of 10% glucose yeast water).

| Temp. C. | After | | | Acidity; c c. of n/10 NaOH to neutralize 10 c.c. of solution |
|---|---|---|---|---|
| | One day. | 2 days. | 4 days. | |
| 10~15° | – | – | ǂ | 10.2 |
| 26~28° | + | ++ | ++ | 51 0 |
| 30~31° | + | ++ | ++ | 47.6 |
| 36° | ++ | ++ | ++ | 31.8 |
| 40~41° | – | – | – | – |

Remarks: + and — denote the presence and absence of the acid. Acidity in the table means the difference of the acidity of culture and that of original medium.

### *The maximum yield of gluconic acid.*

To determine the maximum yield of gluconic acid, yeast water containing 10% of glucose (Merck) was used and incubated at 26~28°C. The acid attained, after 10 or 18 days, to the maximum yield as recorded in the following table.

| Days. | Acidity c.c. n/10 NaOH | Gluconic acid % | Days. | Acidity c.c. n/10 NaOH | Gluconic acid % |
|---|---|---|---|---|---|
| 3 | 14.7 | 2.88 | 13 | 51.2 | 10.03 |
| 4 | 25.8 | 5.05 | 18 | 52.4 | 10.11 |
| 6 | 45.8 | 8.97 | 22 | 51.6 | 10.11 |
| 8 | 50.6 | 10.91 | 30 | 51.2 | 10.03 |
| 10 | 51.8 | 10.15 | 50 | 51.8 | 10.15 |

### *Is it possible the oxydation of acetic acid by this bacillus.?*

To yeast water 3% of ethyl alcohol was added and was seeded with the bacillus prior to the incubation at 26~28°C. The other medium which was added 3% of acetic acid instead of ethyl alcohol, was used for the determination of the loss by mere evaporation. In either case, there was no loss by oxydation of ethyl alcohol, inasmuch as it was under the limit of the loss by evaporation, as shown in the table below:——

| Duration of cultivation, days. | Acidity of each 10 c.c. of the medium | |
|---|---|---|
| | Alcohol added prior to the seeding. | Added acetic acid and no infection of bacillus. |
| 5 | 1.4 | 37.2 |

| | | |
|---|---|---|
| 8 | 1.4 | 34.8 |
| 12 | 26.4 | 35.8 |
| 15 | 25.8 | —— |
| 20 | 26.4 | 34.0 |
| 30 | 25.0 | —— |
| 40 | 21.2 | 33.0 |

### *The production of gluconic acid by authors bacilli and other bacilli already known.*

To determine the maximum yield of gluconic acid by our bacilli glucose (10% Merck)—yeast water was used. The cultures were incubated at 26~28°C during 14 days to obtain their maximum yield.

Bacterium Hoshigaki var. rosea n. sp. gave the most highest yield of gluconic acid among bacilli already known, although our new bacillus viz: Bact. industrium var Hoshigaki excelled over in its yield. The experimental data are summarised below :——

| Bacterium. | Acidity for 10 c.c. of medium expressed by c.c. of n/10 NaOH |
|---|---|
| Bact. industrium v. Hoshigaki: n. sp.(9) | 52. (after 6 days). |
| Bact. Hoshigaki v. rosea n. sp. | 51.2 (after 14 days). |
| Bact. gluconicum(30) | 45.4 |
| Author's bacterium C. | 43.0 (after 14 days). |
| Bact. industrium(10) | 42.8 |
| Author's bacterium B. | 33.0 (after 14 days). |
| Bact. oxydans.(10) | 32.2 |
| Bacillus of Yagi and Hashitani.(31) | 28.0 (after 20 days). |
| Bact. aceti.(10) | 13.4 |
| Bact. acetigenum.(10) | 9.8 |
| Thermobact. aceti.(10) | 8.0 |
| Bact. xylinum.(10) | 6.4 |
| Bact. acetosum.(10) | 5.0 |
| Bact Pasteurianum.(10) | 1.2 |
| Bact. Kützingianum.(10) | 0.8 |

### *Affinities and characters of Bact. Hoshigaki var rosea n. spec.*

This bacterium is nonmotile and its film in fluid medium has no reaction of starch and cellulose. It cause a turbidity in fluid medium but has no power of aciditying maltose. From these points stated it stands very near to Bact. acetosum, Bact. rancens, Bact. ascendens or Bact. gluconic Hermann and by the property of producing abundant quantity of gluconic acid, it should be classed in the same category to Bact. industrium, Bact. oxydans, Bact. gluconicum Hermann and bacillus of Yagi and Hoshitani. Although with such properties, as mentioned, analogous to bacilli already known, yet we have many other specific properties characteristic to identify this as quite new one as tabulated in next page. Among the table we find *a red colour production of this bacillus in wort or "Saké"* and the same in all media when they contain Ca-carbonate in addition to them. This *colour production* is a quite specific properties of this one and none of acetic bacilli already known, has ever do the same. The yield of gluconic acid from glucose is most highest among bacteria ever known.

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(9) On the nature of this bacillus and Bact. B et C. the report will be followed soon.

(10) All bacterium marked with, are quoted from Henneberg's Gährings bakteriologie.

### *The concentration of gluconic acid obtainable in medium by this bacillus.*

Two kinds of the medium were used viz.——

| | | | |
|---|---|---|---|
| (1) | Glucose (Merck) | 8 g. | |
| | Hoshigaki (3%) ext. | 100 c.c. | This contained 9.850 g. glucose in 100 c.c. |
| (2) | Glucose (Merck) | 10 g. | |
| | Yeast water | 100 c c. | |

The culture held at room tep. (Ca. 25°C) during 14 days.

In the second medium, the accumilation of gluconic acid attained already to 79.8% of the total glucose after 6 days from the begining and after 14 days it increased to 97.8% viz. 98.6% of the calculated yield.

The first medium held good as favorable for this organism, as shown in the table below. The yield attained about 90% of the total glucose.

| Medium. | Duration of cultivation. Days. | Glucose. oxydised. g. | Volatile acid c.c. of n/10 NaOH | Gluconic acid. | $\frac{\text{Gluco. Acid}}{\text{Tot. Glucose.}}\times 100$ | $\frac{\text{Glu. a}}{\text{Glucose oxydised.}}\times 100$ | Cal % |
|---|---|---|---|---|---|---|---|
| Hoshigaki Ext. +glucose. | 13 | 8.76 | Trace | 8.903 | 89.0 | 101.6 | 99.1 |
| Yeast-water glucose. | 6 | 7.85 | Trace | 7.983 | 79.8 | 101.7 | 93.5 |
| Same. | 10 | 8.68 | 2.1 | 8.874 | 88.7 | 102.2 | 93.9 |
| Same. | 14 | 9.118 | 4.0 | 9.782 | 97.8 | 107.2 | 98.6 |

N. B. a.) Glucose was determined by Bertrand's method. b.) Volatile acid The steam distillation was applied to the filtered medium and the distillate was titrated with n/10 NaOH solution associated phenolphthalein as indicater. c.) Gluconic acid: The residue of steam distillation was neutralized with Ca-carbonate and evaporated to a thick mass, prior to the development of crystalls of Ca-gluconate, which was washed by spirit (80%) to remove adhered glucose.

| | Bact. Hoshigaki var rosea. n. sp. | Bact. acetosum | Bact. ascendens. | Bact. gluconicum. Hermann | Bact. industrium. | Bact. oxydans. | Bacillus of Yagi et Hashitani. |
|---|---|---|---|---|---|---|---|
| Nature of film. | Very thin film. like powder. Bact. ring ascend to wall of vessel. | White flat film, which forms foulds. Trace of ascending growth. | Like Bact. Hoshigaki. | No film. occurs. | Thick pasty film, which ascend to the wall of vessel. | Thin film, ascend to the wall of vessel. | Fragmental film. |
| Cell form and its size. | Majority are single or pair but chain form seldom occurs, 0.5~0.9μ×0.5~1.45μ. | Long chain form 0.4~0.8μ×1.0μ. | Single or pair Chain form occurs seldom. 0.7~0.9μ×0.9~1.4μ. | Diplo-coccus. | Irregular aggregates or short chain. 0.8~1.2μ×1.6~1.8μ. | Single and its form indefinite Long chain. 0.8~1.2μ×2.4~2.8μ. | Short bacillus, 0.8~1.0×2.3×3.0μ. |
| Involution form. | Involution from with swollen. bulb. some short or long or curved. Gigantic cells. | Spindle shaped threads. | Thread with swollen bulb, are branched Branch is longer than the case of oxydans. | | Occurs seldom. | Threads with swollen bulbs and branches. | Occurs seldom. |
| Starch and cellose reaction of film. | − | − | − | − | − | − | − |
| Motility | − | − | − | − | − | − | − |
| Registance limit againt glucose (%). | 40 | 45 | 35 | 50 | 50 | 35 | 35 |
| The same againt ethyl alcohol (%). | 6~7 | 11 | 12 | 9 vol % | 6~7 | 7 | 6 |
| The same against NaCl (%). | 1.5~2.0 | above 3 | | | 1~3 | 0.3 | |
| The same against acetic acid (%). | 1.5~2.0 | above 2 | 2~3 | | 1.5~2.0 | 1.5~2.0 | |
| Maximum concentration of acetic acid formed %. | 1.8 | 6.6 | 9.1 | 1.6 | 2.7 | 2.0 | 0.38 |
| Yield of propyric acid formed (2.0% propyl alcohol.) | 1.66 | 1.8 | 1.9 | | 1.46 | | |
| Maximum concentration of gluconic acid produced %. | 17.2 | 4.6 | | | 16.6 | 8.0 | 12~13 |
| Optimum temp. for growth. C. | 30~35°C | 28° | 34° | | 23° | 12~21° | |
| Oxydation of acetic acid. | ± | + | + | | − | − | |
| Production of colouring mater. | Red colouring matter is formed, in wort, "Sak'e or any nutrient medium containing Ca-carbonate. | − | − | − | − | − | − |
| Assimilation of Am-salts | − | + | + | + | | | |
| Acid formation from Arabinose | + | − | − | + | + | + | + |
| Glucose. | + | + | + | + | + | + | + |
| Galactose. | + | + | − | + | + | + | + |
| Fructose. | (+) | − | − | + | + | + | + |
| Saccharose. | − | − | − | + | + | + | + |
| Maltose. | − | − | − | − | + | + | (+) |
| Lactose. | − | − | − | − | + | (+) | + |
| Raffinose. | − | − | − | + | + | + | |
| Dextrine. | − | − | − | (+) | + | + | + |
| Starch. | − | − | − | − | + | − | (+) |
| Inulin. | − | − | | − | − | − | |
| Methyl-alcohol. | − | − | − | − | (+) | − | − |
| Ethyl-alcohol. | + | + | + | + | + | + | + |
| Propyl-alcohol. | + | + | + | + | + | + | |
| Glycerine. | + | − | − | + | + | + | − |
| Mannite. | + | − | − | (+) | + | + | + |
| Sorbite. | − | | | | | | |

### *The yield of gluconic acid and the nature of nitrogen source.*

For gluconic acid fermentation of Bact. Hoshigaki var rosea., yeast-water contribute very good nitrogen source as stated above, but we need some other source of nitrogen in practical use of this fermentation. For this purpose "Hoshigaki" extract, soy bean extract and extract of rice bran were tested. As the result, in the case of "Hoshigaki" extract, the most favourable act was found when it gave 0.0077 g. of nitrogen to 100 c.c. of the medium and obtained as such 0.0196 g. in the case of soy bean extract, while the extract of rice bran contributed the most inferior source of nitrogen as we have gained lower yield of acid even in addition of 0.1318 g. of nitrogen to 100 c.c.

### The preparation of extracts.

(1) "Hoshigaki" extract. To "Hoshigaki" ten times of water in quantity was added and warming in water bath several hours, adding water frequantly to recover the loss by evaporation.

The extract show 4° of Balling, containing 3 g. of glucose and 0.0231 g. of nitrogen in 100 c.c. To this extract 8 g. glucose (Ozawa) was added to 100 c.c.

(2) Soy-bean extract. To soy-beans two times of water in quantity was added and warmed in water bath as extract (1). The extract (8°B) contained 0.1178 g of nitrogen in 100 c.c. This was diluted in several ways to get many media with different contents of nitrogen, adding 13 g. glucose (Ozawa).

(3) Rice bran extract. It was prepared in quite same way as soy-bean extract.

It gave 2° of Balling, and contained 0.0659 g. of nitrogen in 100 c.c. of it. A part of the extracts was evaporated to prepare more higher contents of nitrogen. Glucose was added as in the case of soy-bean extract.

The cultures were incubated at 26~28°C during 18 days. The results are recorded in the table below:—

(I) "Hoshigaki" extract (100 c.c.)

| No. | Glcose. g. | Nitrogen. g. | Volatile acid c.c. of n/10 NaOH. | Non. Vol. acid c.c. of n/10 NaOH. | Glucon. calcu. g. | Ca-gluc. | | $\frac{\text{Glucon. a.}}{\text{Tot. Sugar.}}\times 100$ |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Calc. g. | found. g. | |
| I. | 7.65 | 0.0694 | 4.0 | 210.0 | 4.11 | 4.68 | 5.0 | 53.7 |
| IV. | 8.35 | 0.0115 | 4.7 | 405.3 | 7.94 | 9.05 | 9.4 | 95.0 |
| V. | 8·22 | 0.0077 | 4·0 | 430 0 | 8·42 | 9·59 | 9·6 | 102 4 |

(II) Soy-bean extract. (100 c.c.)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| I. | 8.65 | 0.1178 | 13.2 | 397.3 | 7.79 | 8.88 | 9.0 | 90.0 |
| III. | 8.65 | 0.0392 | 8.7 | 409.9 | 7.93 | 9.04 | 9.1 | 91.6 |
| V. | 8·65 | 0.0196 | 4·8 | 456·2 | 8·94 | 10.10 | 10.0 | 103·4 |

(III) Rice bran extract. (100 c.c.)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| I. | **8.65** | **0.1318** | **4.5** | **388.5** | **7.41** | **8.44** | **8.7** | **85 6** |
| II. | 8.65 | 0.0659 | 3.1 | 357.9 | 7.01 | 7.99 | 8.2 | 81.0 |
| V. | 8.65 | 0.0109 | 3.0 | 220.0 | 4.31 | 4.91 | 5.1 | 49.8 |

N. B. If we increase the contents of nitrogeu in rice bran extract, we can expect better yield than tabulated.

The quantity of glucose in the table is the result of quantitative determination prior to the experiment. The methods of determination of acids are quite same as mentioned above.

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# ON GLUCONIC FERMENTATION. PART II.

## ON THE FORMATION OF OXY-GNUCONIC ACID. (5-KETO-GLUCONIC ACID).

By

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In 1886 Boutrox(1) found a new organic acid from fermentation product of his Micrococcus oblongus, which was cultured in the yeast water contaning glucose or gluconic acid associated with Ca–carbonate in either case. The acid, having a ketonic radical and laevo-rotatory power with the formula of $C_6H_{10}O_7$ or assumed constitutional formula of $CH_2OH{\cdot}CO{\cdot}OH{\cdot}CH{\cdot}CH{\cdot}OH{\cdot}OH{\cdot}CH{\cdot}COOH$ was named oxy–gluconic acid ($\alpha$–$\beta$–$\gamma$–$\omega$ tetroxy–$\delta$–oxo–pentan–$\alpha$ –carbonic acid).

Bertrand's memoir(2) about this acid, as the product of his sorbose bacteria from gluconic acid at 18~25°C, was about a decade later from pioneer's work.

Ruff's(3) chemical preparation of this acid from Ca-gluconate by oxidising with bromine or hydrogenperoxide in presence of basic ferric acetate was unsatisfactory, as its yield was limitted near 1%, giving rise many byproducts such as arabinose, glycollic acid and formic acid. On the other hand, Killiani(4) prepared the same in the most purest state from glucose by oxidising it with nitric acid. The yield was better than former as such 15~16%.

Quite recently, S. Hermann(5) in the study of his Bact. gluconicum found about 70% of 5–keto–gluconic acid from glucose, gluconate, free gluconic acid and fructose in the culture of three months old. In the same year K. Bernhauer aud K. Schön(6) took advantage to find out the same acid from glucose in the culture of Bact. xylinum in presence of Ca–carbonate.

In the former publication(7) of authors on gluconic fermeatation, thers was mentioned four new acetic bacteria and one of them viz. Bact. Hoshigaki var rosea was described in details. The other three varieties, including Bact. industrium var Hoshigaki, happened to give rise oxygluconic acid from glucose in presence of Ca–carbonate.

## Experimental.

To 100 c.c. of yeast water*, 10g. of glucose (Merck) and 2.5g. of Ca–carbonate was added and the three varieties of authors' bacteria were seeded, provided the necessary pasteurisation of the the medium.

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* To 50 g of pressed beer yeast, 1000 c.c. of tap water was added and taken in water bath heated to boiling during two hours. 10 c.c. of it wanted 0.6 c.c. of n/10 of NaOH.

The cultures were incubated at 26~28°C and after 20 days, the carbonate added in the flask completely dissapeared, especially in the culture of Bact. industrium var Hoshigaki, owing to the formation of some acid. After that, standing these cultures at room temperature (18~20°C) for sevesal days, plentiful crystals developed covering the whole surface of the bottom of flask.

### *Isolation and identification of the acid.*

The crystals of salt, with the shape of oblique plates or prisms, obtained from the culture of Bact. industrium var Hoshigaki, were washed several times with distilled water prior to the drying at 100°C. The acid under examination was free from nitrogen and its salt gave just calcium as the ash on ignition.

The properties shown below are given by the free acid thus : ——

(1) It reduces Fehling's solution gradually even at room temperature, but suddenly upon warming.

(2) It reduces ammoniacal silver nitrate when warmed.

(3) Sodium nitroprusside in the presence of ammonia, when warmed gives a dark yellow green colouration to the acid under examination.

(4) Sodium nitroprusside, warmed in presence of caustic potash with the acid under examination, gives a red colouration, which changes to green when acidified by acetic acid.

(5) The solution of neutral salt of the acid under examination, waring after the addition of ferric chloride, takes a red colour which changes to yellow by cooling,

(6) It gives Bial's orcin reaction(7) and Wheeler and Tollen's phloroglucine reaction(8) for pentose.

(7) It gives furfurol when digested with concentrated HCl. The furfurol after distillation is proved by well known colour reaction by anilin acetate or barbituric anilin acetate.

(8) Seliwanoff's(9) reaction which is specific one for ketose, is positive by this acid.

From these reactions given above, the conclusion may be drown most safely that the acid under examination should be one of the ketonic acid with six carbon atoms in the molecule. Further, its specific rotatory power is −14.3° and it dose not form a crystalline lactone, although it has been evaporated at low temperature under reduced pressure.

It colours brown by simple warming, but in presence of alkali decomposes changing to a dark brown mass. It dissolves easily in water and alcohol, but almost insoluble in ether. These characteristics remind us Boutroux's oxygluconic acid and its identification follows from the description below.

(1) *Specific rotatory power of the acid.* Free acid rotated the polarized light 14.3° to the left, thus :——

$C=9.76 \qquad l=0.5\text{ dm.} \qquad t=18C \qquad \alpha=-0.70°$

$$[\alpha]^{18°}=\frac{\alpha\times100}{C\times l}=\frac{-0.7\times100}{9.76\times0.5}=-14.3°$$

After Ruff(10) $[\alpha]_D=-10.3°$ (2% Ca-salt with HCl)

$[\alpha]_D>-12.8$ (free acid)

After Boutrox(11) $[\alpha]_D=-14.5°$ ($C=2$)

(2) *Calcium and the water of crystallization of Ca-salt.* The Ca-salt was obtained from aqueous solution of potassium salt by the addition of quantitative necessary Ca-acetate. It appears as beautiful oblique prism or plate, which dried at 100°C give 8.26% of calcium shown below :——

Substance taken 0·6269 g. $CaCO_3=0.1259$ g. $Ca=0.0518$ g.

| | | |
|---|---|---|
| Calc. as $(C_6H_9O_7)_2$ Ca+3$H_2O$ | Ca. | 8.34% |
| Found. | 〃 | 8.26% |

Thus the water of crystallization of the salt must be three molecules and it was substanciated by drying the salt at the temperature of 100~140°C.

The results are given below :——

| Temp. C. | Substance. g. | Decrease by weight g. | Loss of water. % | Calc. as | Water of crystallization. % |
|---|---|---|---|---|---|
| 100 | 0.5181 | 0 | 0 | | |
| 100~115 | 0.5107 | 0.0074 | 1.42 | $(C_6H_9O_7)_2Ca.\ 1H_2O$ | 3.78 |
| 120~130 | 0.4793 | 0.0388 | 7.49 | $(C_6H_9O_7)_2Ca.\ 2H_2O$ | 7.56 |
| 130~140 | 0.4596 | 0.0585 | 11.30 | $(C_6H_9O_7)_2Ca.\ 3H_2O$ | 11.34 |

N. B. The substance at 120~130°C gained somewhat yellow color and at 130~140°C changed to brown, indicating the decomposition although in trace.

The calcium salt dehydrated contained 9.46% of calcium, identifying to that of Ruff's(12) and Boutroux's(13) Ca-salt of oxygluconic acid.

(3) The result of *elementary analysis of the Ca-salt* is described below.

| Substance taken 0.2200 g. | $CO_2=0.2411$ g. | $H_2O=0.1014$ g. |
|---|---|---|
| | C. | H. |
| Calc. as $(C_6H_9O_7)_2Ca+3H_2O$ | 29.98% | 5.03% |
| Found. | 29.88% | 5.12% |

(4) *Strontium salt.* Strontium salt was prepared by the addition of strontium acetate to the aqueous solution of potassium salt.

It crystallized in oblique prism and after recystallizing from hot water, was dried at 100°C prior to the determination of strontium. The result is shown below :——

| | |
|---|---|
| Sabstance taken | 0.3190 g. |
| $SrSO_4$=0.1191 g. | Sr=0.0568 g. |
| Sr calc. as $(C_6H_9O_7)_2SrH_2O$ | 17.81% |
| Found. | 17.80% |

Thus, the water of crystallization of this salt seems to be just one molecule, showing discrepancies to the result of Boutroux[14], whose Sr-oxygluconate contained 3 or 1½ molecules of water of crystallization.

(5) *Lead salt.* This salt was obtained from its potassium salt by additein of lead acetate. The salt dried at 100°C, contained no water of crystallization as shown below :——

| | |
|---|---|
| Substance taken | 0.3380 g. |
| $PbSO_4$=0.1737 g. | Pb=0.1186 g. |
| Found. | Pb 35.09% |
| Calc. as $(C_6H_9O_7)_2Pb$ | Pb 34.92% |

Lead salt of Boutroux's(15) oxygluconic acid contained 1 or 2 molecules of water of crystallization.

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# ON THE NATURAL PIGMENTS OF RAW SILK FIBRE OF THE DOMESTIC COCOON. (PART II)

## XANTHOPHYLL OF MULBERRY LEAVES AS A SOURCE OF YELLOW COCOON XANTHOPHYLL.

By

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(Received November 4 th., 1930).

In previous papers I have reported the occurrence of xanthophyll in the domestic yellow cocoon and the coincidence of this pigment with that of plant origin, formerly studied by Willstätter and others. I have also discussed there that xanthophyll of the yellow cocoon should be probable to be derived biologically from that of mulberry leaves upon which the silk worms feed.

In this research I have isolated xanthophyll from mulberry leaves in a good yield and identified it as wholly coincides with that of yellow cocoons.

## Experiments.

1 kg. of fresh mulberry leaves (plucked off on 6 th, Sept.) was finely chopped and soaked in 5 L. of 90% ethyl alcohol in a large beaker and digested under 15 lbs. pressure for half an hour in an autoclave, about 60% alcohol was taken as a boiling medium, instead of water. After filtration on a Büchner funnel, the macerated leaves were further treated with boiling alcohol and filtered as before and then washed with warm alcohol until the macerated leaves became almost colorless. All the alcoholic extract of the chloroplast pigments thus obtained was concentrated under reduced pressure, passing carbon dioxide to avoid the oxidation of the pigments. To the condensed pigments suspension, ether added and chlorophyll and carotinoids were transferred to the ethereal layer. After condensation and complate saponification with methyl alcoholic potash, the ether solution was washed with water to remove potassium chlorophyllines completely. Ether solution was then dehydrated by anhydrous sodium sulphate and evaporated to dryness, upon which 600 c.c. of petroleum ether (b.p. 40~60°) was added and stirred well. Thus crude xanthophyll was precipitated, dissolving carotin and waxy substances in petroleum ether fraction.

Boiling this crude xanthophyll with low petroleum ether, pure crystalline xanthopoyll was obtained by recrystallisation from boiling methyl alcohol.

The yield was 0.165 g. viz. 0.044% of the anhydrous sample which showed about 73% of the theoretical value. (Colorimetric determination.)

In another experiment I have obtained 0.142 g. of crystalline xanthophyll from 500 g. of air dried mulberry leaves, (10.25% moisture content) which showed in yield 73% of that, obtained from fresh leaves. Thus, xanthophyll in the green fresh leaves was considered to be oxidised during the air drying. Therefore, the preparation must be recommended to start from fresh leaves.

The crystalline xanthophyll thus isolated showed very similar coloration and crystal form with that, isolated from yellow cocoon and proved its melting point to be 175° (uncorr.) which revealed no change by mixed probe with cocoon xanthoyhyll.

| | | |
|---|---|---|
| Elementary analysis :— | C% 84.24 | H% 10.08 |
| Molecular weight :— | 537.2 | |

The absorption bands of the alcoholic solution of this xanthophyll coincides wholly with that of cocoon xanthophyll.

Judging from the results obtained, the origin of xanthophyll in the domestic yellow cocoon can be asked in the mulberry leaves upon which the silk worms feed.

The new extraction method of chloroplast pigment from fresh green leaves, adopted in the above experiment, can be considered to be recommended widely.

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# STUDIEN ÜBER DIE GÄRUNGSPRODUKTE DER SCHIMMELPILZE.

## VIII. MITTEIL. ASPERGILLUS GLAUCUS. (III).

von

YUSUKE SUMIKI.

*(Aus dem Laboratorium der Agrikulturchemie an der Staats-Universität zu Tokyo).*

(Eingegangen am 18 November 1930).

In früheren Mitteilung (Bull. agrikul. chem. Soc. Japan, **5**, 10, 1929) wurde ein Verfahren beschrieben, dass bei der Kurtur von Asp. glaucus die

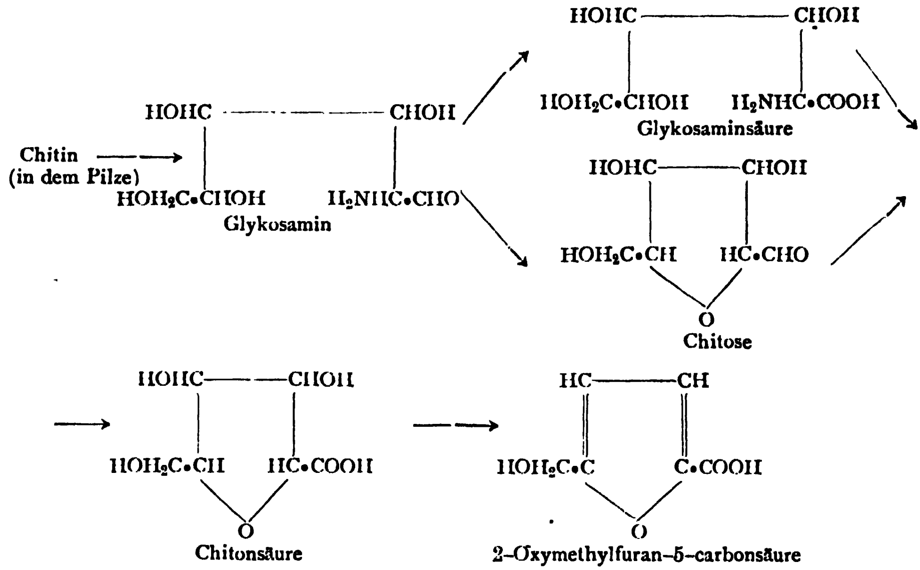
2–Oxymethylfuran-5-carbonsäure in kleinen Menge gebildet ist und identifiziert wird.

In diesem Bericht wird die Mechanik von der Produktione der 2–Oxymethylfuran–5–carbonsäure erklärt. Mann kann vorschlagen folgende drei Mechaniken.

(1) Glykose→Glykonsäure→(Chitonsäure→)2–Oxymethylfuran–5–carbonsäure.

(2) Glykose——→Oxymethylfufurol——→2–Oxymethylfuran–5–carbonsäure.

(3)



Bei allen Zuchtungen von Asp. glaucus in der Glykonsäure oder Oxymethyl–furfurol erhaltende Nährlösungen als Kohlenstoffquelle wird die 2–Oxymethylfuran–5–carbonsäure nicht gewonnen. Aber bei der Kurturen von Asp. glaucus in der Nährlösungen, welche Glykosamin, Glykosaminsäure, Chitose oder Chitonsäure erhalten sind, wird die 2–Oxymethylfuran–5–carbonsäure immer gewonnen und identifiziert. Ueberdies wird die Chitonsäure aus Glykosamin und Chitose mit der 2–Oxymethylfuran–5–carbonsäure mittels des Pilzes gebildet. Es zeigt sich deutlich, dass die erste und zweite Mechaniken irrig sind und nur der dritte rechtig ist.

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